VOLUME 37

ARCHIVES OF PATHOLOGY

MARCH 1944

PUBLISHED MONTHLY BY AMERICAN MEDICAL ASSOCIATION, 535 NORTH DEARBORN STREET, CHICAGO 10, ILLINOIS. ANNUAL SUBSCRIPTION

Entered as Second Class Matter Jan. 26, 1926, at the Post-office at Chicago, Under the Act of Congress of March 3, 1879.

CONTENTS OF PREVIOUS NUMBER

ARCHIVES OF PATHOLOGY

VOLUME 37 MARCH 1944 NUMBER 3

FLUORESCENT MATERIAL (CEROID) IN EXPERIMENTAL NUTRITIONAL CIRRHOSIS

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In their studies of experimental dietary injury of the liver (necrosis and cirrhosis) in rats, György and Goldblatt [1] observed, in unstained sections, peculiar light greenish yellow globules of variable size and shape, mainly enmeshed in periportal fibrous connective tissue. They found that the globules did not give the reaction for iron, were not dissolved out by organic solvents for fats and, in frozen sections, gave a positive oxidase reaction. The material reacted as a lipid with various stains for lipids, in paraffin as well as in frozen sections. By its insolubility in solvents for lipids it differed from most other known types of lipids, so it was regarded as an intimate lipid-protein combination probably originating in degenerated or necrotic liver cells. Lillie, Ashburn, Sebrell, Daft and Lowry [2] found the same material in the livers of rats with dietary cirrhosis and described it as a basophilic, acid-fast, hyaline substance, to which they gave the name "ceroid." They observed some ceroid in Kupffer and liver cells, in trabeculae and pulp of the spleen, in phagocytic cells of lymph nodes and in the adrenal glands, bone marrow and the lungs. In a study devoted to the influence of alcohol in experimental cirrhosis, the amount of ceroid present was considered [3] a good indicator of the degree of cirrhosis. Pigment with similar staining reactions was found in connective tissue strands of cirrhotic rat livers by Blumberg and co-workers [4]; they considered it to be hemofuscin. Edwards and White [5] also found a canary yellow pigment in rats fed with butter yellow (p-dimethylaminoazobenzene) in which hepatic tumors had developed. On the basis of various staining reactions they concluded that it was not hemofuscin, but a lipoid substance in conjugated form. In a subsequent paper [6] they considered that pigment to be derived from disintegrated hepatic tissue. They showed that it was not produced by the feeding of butter yellow but by the basal diet. Deposition of a similar pigment was also found in mice in which cirrhotic changes with hepatoma formation had been produced with carbon tetrachloride [7] and with orthoaminoazotoluene,[8] but György and Goldblatt, who examined rat livers cirrhotic from carbon tetrachloride,[9] failed to discover any ceroid.

Examination of sections of livers from rats with experimental dietary cirrhosis (György and Goldblatt) under the fluorescence microscope (Popper) revealed golden brown fluorescent material which in size and distribution appeared identical with the globules (ceroid) which had been demonstrated by special stains and solubility properties.[10] Since this specific method for the demonstration of ceroid permits a further investigation of its incidence and development, the livers of a larger series of rats with experimental cirrhosis have been examined under the fluorescence microscope, and this is a report of the study.

From the Hektoen Institute for Medical Research of the Cook County Hospital, Chicago (Dr. Popper), the Babies and Childrens Hospital and the Department of Pediatrics and the Institute of Pathology of Western Reserve University School of Medicine, Cleveland (Drs. György and Goldblatt).

1. György, P., and Goldblatt, H.: J. Exper. Med. **75**:355, 1942.

2. Lillie, R. D.; Ashburn, L. L.; Sebrell, W. H.; Daft, F. S., and Lowry, J. V.: Pub. Health Rep. **57**: 502, 1942.

3. Lowry, J. V., and Ashburn, L. L.: Quart. J. Stud. on Alcohol **3**:168, 1942.

4. Blumberg, H., and Grady, H. G.: Arch. Path. **34**:1035, 1942. Blumberg, H., and McCollum, E. V.: Science **93**:598, 1941.

5. Edwards, J. E., and White, J.: J. Nat. Cancer Inst. **2**:157, 1941.

6. White, J., and Edwards, J. E.: J. Nat. Cancer Inst. **3**:43, 1942.

7. Edwards, J. E., and Dalton, A. J.: J. Nat. Cancer Inst. **3**:19, 1942.

8. Andervont, H. B.; Grady, H. G., and Edwards, J. E.: J. Nat. Cancer Inst. **3**:131, 1942.

9. This material was obtained from Dr. Robert Heinle, of the Department of Medicine at the University Hospitals, Western Reserve University, Cleveland.

10. The methods of staining with methyl green for the demonstration of ceroid were developed and carried out by Miss Ethel Lieb, assistant curator of the Museum of the Institute of Pathology, Western Reserve University School of Medicine, who supplied the following details of the methods:

(Footnote continued on next page)

MATERIAL AND METHODS

The material of this study is composed of the livers of 287 rats which were kept for fifty-nine to two hundred and twenty-eight days on the synthetic diets to be described. Most of the animals were killed after one hundred and fifty days. All rats received supplements of fractions of crystalline vitamin B, namely, 20 micrograms of thiamine, 25 micrograms of riboflavin, 20 micrograms of pyridoxine and 100 micrograms of calcium pantothenate daily. Certain groups of the rats received, respectively, special supplements related to the development of nutritional cirrhosis (cystine, choline, p-dimethylaminoazobenzene [so-called butter yellow]).

Frozen sections of liver were examined in some instances. For the great majority of the rats, only paraffin sections were studied. Frozen and paraffin sections were examined under the fluorescence microscope by use of a technic and filters described previously.[11] Frozen sections of liver fixed in 0.85 per cent solution of sodium choloride containing formaldehyde in the concentration of 4 per cent were stained with the water-soluble fluorescent fat stain, phosphin 3R. It produced a silvery white fluorescence of the neutral fat in a brown background.

A. Frozen Sections of liver fixed in 0.85 per cent solution of sodium chloride containing formaldehyde in the concentration of 4 per cent:

1. Collect frozen sections in water.

2. Stain two minutes in 20 per cent alcohol containing methyl green in the concentration of 0.5 per cent.

3. Rinse in water or in 1 per cent acetic acid followed by water and mount in gum arabic (acacia).

B. Paraffin Sections of liver fixed in 0.85 per cent solution of sodium chloride containing formaldehyde in the concentration of 4 per cent:

1. Paraffin sections of the formaldehyde-fixed material are carried down to water as usual.

2. Stain five minutes in 20 per cent alcohol containing methyl green in the concentration of 0.5 per cent. (Make fresh or, as solution gets older, increase staining time.)

3. Rinse in water or in 1 per cent acetic acid followed by water and mount in gum arabic.

If it is desired to mount the section in clarite (a resin; see Lillie, R. D.: Stain Technol. **16**:127, 1941), proceed as follows: Stain overnight in the following mixture: 5 cc. of the 0.5 per cent methyl green solution diluted to 50 cc. with 4 per cent solution of formaldehyde, chemically pure. Rinse in water and place in 10 per cent phosphotungstic acid for five hours. Rinse in water and stain for three minutes in Van Gieson solution. Dehydrate rapidly in absolute alcohol, clear in terpineol-xylene (75 per cent terpineol, 25 per cent xylene) followed by two changes of xylene. Mount in clarite.

C. Combined methyl green and fat stain of liver fixed in 0.85 per cent solution of sodium chloride containing formaldehyde in the concentration of 4 per cent:

1. Stain frozen sections in Herxheimer's solution (saturated solution of sudan IV in equal parts of acetone and 70 per cent alcohol) for five minutes after dipping quickly in 70 per cent alcohol.

2. Rinse quickly in 70 per cent alcohol and then in water.

3. Stain two minutes in 20 per cent alcohol containing methyl green in the concentration of 0.5 per cent.

4. Rinse in 1 per cent acetic acid.

5. Rinse in water and mount in gum arabic.

NOTE: Use the certified brand of methyl green dye, manufactured by National Aniline & Chemical Co.

11. Popper, H.: Arch. Path. **31**:766, 1941.

RESULTS

Before the conditions which promote or interfere with the deposition of ceroid are subjected to analysis, the appearance of this substance under the fluorescence microscope and its development will be discussed.

Experimental Tissue under Fluorescence Microscope.—A great number of the rat livers examined presented bright yellow to golden brown fluorescent globules. The size of these varied from 5 to 25 microns. The degree of fluorescence also varied, even in globules located in the same cell. In visible light the fluorescent material often revealed a yellow to green color and strong refraction. However, in visible light most of the fluorescent globules were hardly recognizable in sections stained with hematoxylin-eosin.

The golden brown fluorescence was stable under ultraviolet rays and was thus easily differentiated from the green fluorescence of vitamin A, which fades under these rays. Prolonged fixation did not interfere with the golden brown fluorescence, in contrast to most other observations on fluorescent details in sections of tissue. Treatment with organic solvents for fat (for instance, exposure to acetone up to seventy-two hours) did not change the fluorescence. The process of embedding in paraffin did not remove it. Since this fluorescence is strong enough to surpass the dim eosin fluorescence, paraffin sections stained with hematoxylin-eosin can be used for the study of ceroid fluorescence.

Reducing agents, or trypsin, did not decrease the fluorescence. Exposure to hydrogen peroxide for as long as seventy-two hours increased the degree of fluorescence of ceroid slightly but also enhanced the fluorescence of the fat. Treatment with acid alcohol slightly decreased the fluorescence. In sections stained with phosphin 3R the fat droplets gave a silvery white fluorescence, whereas ceroid showed the same golden brown fluorescence as in unstained sections. Some fat droplets showed a transitional shade between the silvery white of the fat and the golden brown of the ceroid. If the silvery white fluorescence of fat had been removed by extraction with acetone, the light brown fluorescence of the last-mentioned fat droplets remained, without the white hue; whereas the ceroid fluorescence remained unchanged. In frozen sections the

ceroid globules in the periportal field were often found surrounded by many fine fat droplets in connective tissue and Kupffer cells. These fat droplets occasionally gave strong vitamin A fluorescence.

In sections stained by the Ziehl-Neelsen method the golden brown fluorescent granules were acid fast. They also gave the other histologic reactions characteristic for ceroid, did not give the reaction for iron and were not doubly refractile in polarized light.

droplets of various size were seen. In other livers, with minor pathologic changes, ceroid was recognizable in only a few Kupffer cells, in areas with pronounced edema. The fine droplets outlined the characteristic wing form of the cells. Fat droplets were not seen in them. Sometimes ceroid appeared simultaneously in Kupffer cells and epithelial cells (fig. 1 *A* and *B*). In livers with marked fat infiltration but with moderate connective tissue proliferation, ceroid accumulated in poorly defined areas around the connec-

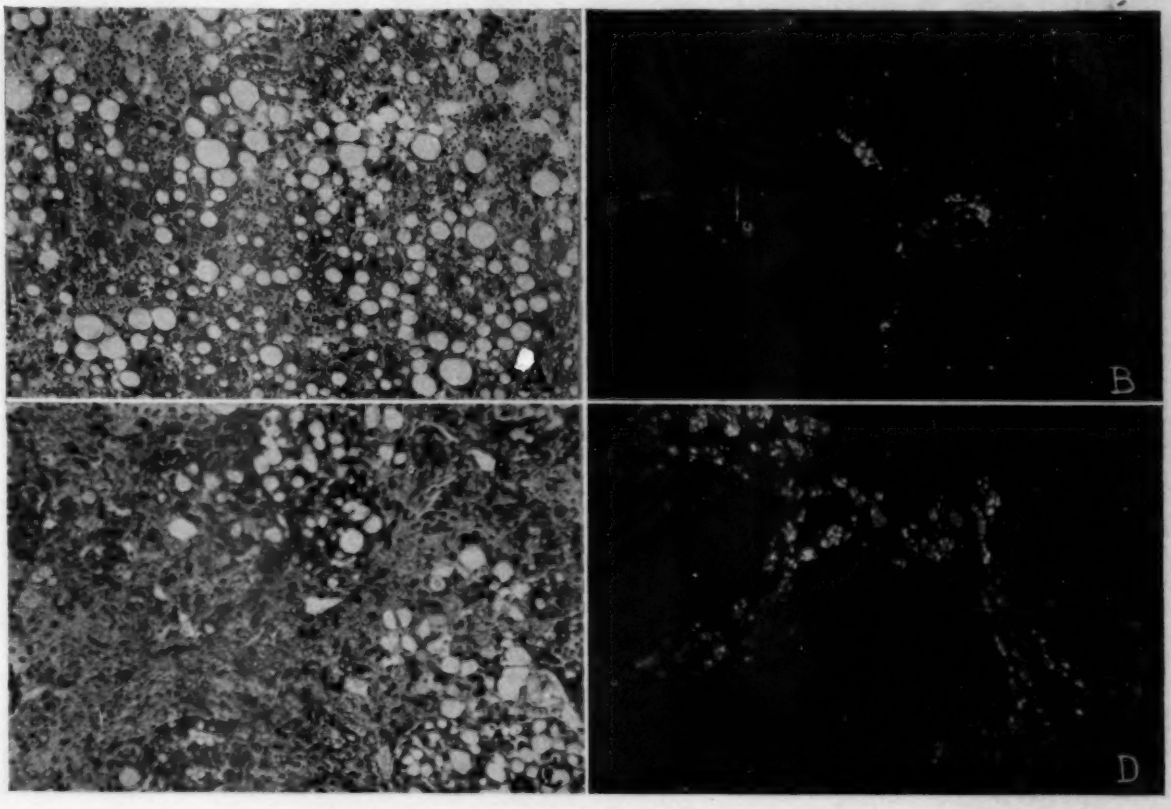

Fig. 1.—*A*, photomicrograph in visible light of liver from a rat kept on a cirrhosis-producing diet. Marked fatty infiltration is seen.

B, fluorescence photomicrograph of the section shown in *A*. It reveals ceroid deposits in clusters of liver cells with fatty changes and in some scattered Kupffer cells.

C, photomicrograph in visible light of liver from a rat kept on a cirrhosis-producing diet. Fatty infiltration and regeneration of the liver cells are shown with marked proliferation of connective tissue in which histiocytes are embedded that contain strongly refractive globules.

D, fluorescence photomicrograph of the section shown in *C*, showing fluorescent ceroid globules in histiocytic macrophages in the strands of proliferated connective tissue.

Development of Ceroid.—In some instances, usually in livers with fatty changes but without marked cirrhosis, ceroid was found only in scattered liver cells. Most of these cells contained several fat droplets in addition to the droplets with ceroid fluorescence. In other instances, in which fat infiltration was more intense, clusters of liver cells filled with ceroid

tive tissue strands in the central and peripheral zones of the lobules. It was present in Kupffer cells, but more was present in the epithelial cells, which appeared pale in visible light because of the storage of ceroid, and in histiocytic mesenchymal cells. The differentiation of the ceroid-laden epithelial cells in the periphery of the lobule from the mesenchymal cells, which looked like

pseudoxanthomatous or foam cells, in visible light was difficult and occasionally impossible. The cytoplasm of both cell types revealed strongly refractile fine vacuoles in visible light. Only the distribution around sinusoids and the arrangement of the connective tissue fibers indicated the nature of the cells (fig. 2 *B* and *C*). Close to the enlarged periportal field the smaller ceroid droplets seemed to become confluent, forming larger droplets. Some livers, with marked proliferation of connective tissue, formation of pseudolobules and regeneration of liver cells, usually associated with fat infiltration, showed deposition of ceroid throughout the lobules. The ceroid foam cells were either isolated between sinusoids and liver cell cords or appeared in clusters. Around the isolated foam cells or between the clusters connective tissue fibers were seen. Regenerated liver cells were free of ceroid.

In places the foam cells accumulated in the form of large nodules, which were visible even in the gross. These nodules were found not only in cirrhotic livers but also, as subcapsular nodules, in livers which otherwise showed only limited pathologic changes. They contained, besides foam cells surrounded by a few connective tissue fibers, large epithelioid cells without ceroid fluorescence. The cytoplasm of the mesenchymal cells was homogeneously light red in sections stained with hematoxylin-eosin. The nodules often contained isolated regenerated liver cells, with giant nuclei and nucleoli, and many capillaries, with proliferated endothelial cells, some of them greatly dilated and filled with plasma but without red cells (fig. 2 *A* and *B*).

The picture most commonly seen was ceroid in the form of globules in large mononuclear cells in well demarcated bands or strands of connective tissue, with occasional round cell

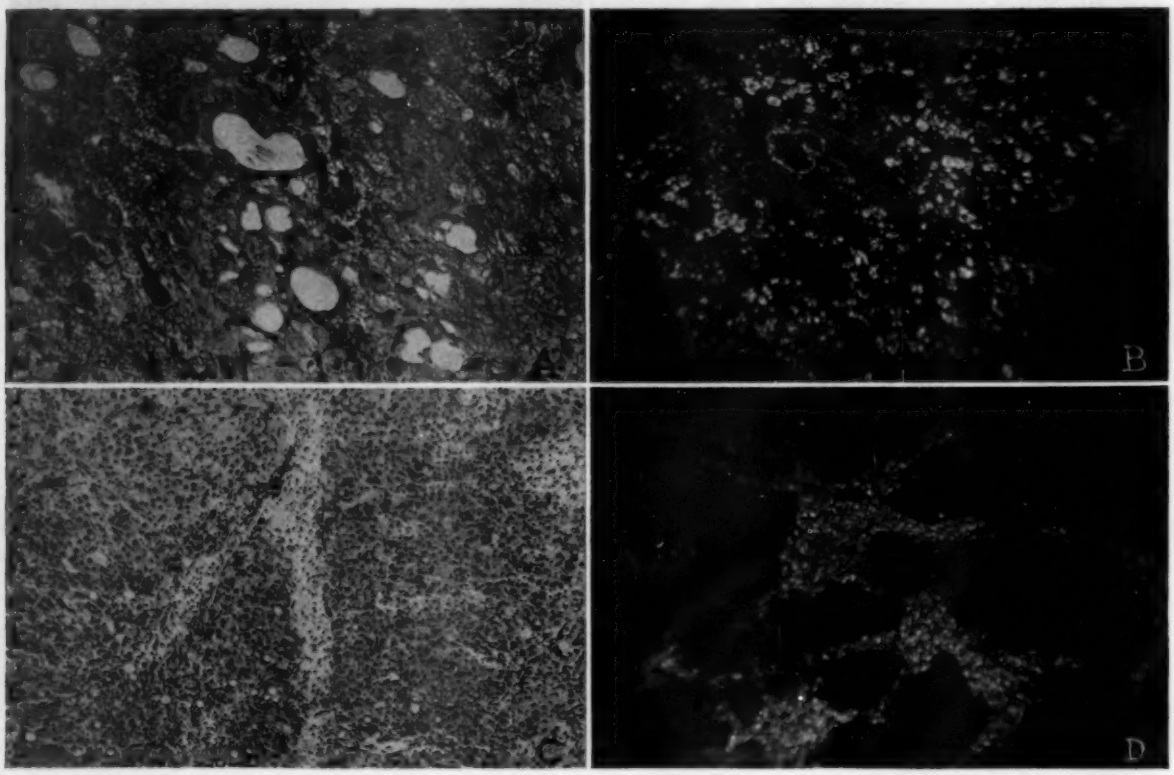

Fig. 2.—*A*, photomicrograph in visible light of liver from a rat kept on a cirrhosis-producing diet, showing part of a nodule composed of large mesenchymatous cells some of which contain deposits of strongly refractive globules. Between them are regenerating large liver cells and dilated capillaries.

B, fluorescence photomicrograph of the section shown in *A*. A greater part of the macrophages contain fluorescent ceroid.

C, photomicrograph in visible light of liver from a rat kept on a cirrhosis-producing diet. Wide strands of proliferated connective tissue separate the lobules, the parenchyma of which fails to show significant pathologic changes. In the strands histiocytes with ceroid are seen.

D, fluorescence photomicrograph of the section shown in *C*, showing extensive ceroid deposits in the connective tissue strands.

infiltration. These strands connected the enlarged periportal and central fields or were below the capsule. Sometimes only a few scattered foam cells were seen in the meshes of the strands. In other instances the strands contained many ceroid foam cells (fig. 2 *C* and *D*). The ceroid in those strands revealed a dark brown fluorescence and appeared dark green and strongly refractile in visible light. Sometimes, usually in large ceroid globules, the color in visible light was so intense that no fluorescence was evident because the strongly colored material screened out the ultraviolet light. In this group the liver parenchyma sometimes showed marked fat infiltration. Often it appeared almost normal (fig. 2 *C* and *D*).

There was great variation in the distribution of ceroid, and different sections of the same liver presented different pictures.

In general, only small amounts of ceroid were encountered in livers in which only necrosis or hemorrhage was present; it was never found close to necrotic or hemorrhagic foci. It was usually absent from cirrhotic livers in which marked cellular infiltration of the portal spaces or bile duct proliferation was seen. It was rarely found, and in only small amounts, in livers in which, following administration of p-dimethylaminoazobenzene, cirrhosis with hepatic tumors of the character of liver cell adenoma or cholangioma had developed.

Influence of Diet on Development of Ceroid.—The amounts of ceroid present in the livers examined were roughly estimated. They are indicated in table 1 by plusses (plus-minus to 4 plus) and correlated with the diets employed and the supplements administered. In table 1 the average amount of ceroid and the average degree of other pathologic changes are recorded for each group of animals on a cirrhosis-producing low casein (low methionine) diet. In this and the following table the degree of cirrhosis was estimated by the extent of proliferation of connective tissue, the infiltration of the portal spaces, the regeneration of liver cells and bile ducts and the degree of reconstruction of the lobular pattern. Animals with pronounced cirrhosis showed jaundice, ascites, anemia and hypoproteinemia, as described by Goldblatt and György.[1]

TABLE 1.—*Average Amounts of Pathologic Change and Ceroid Deposition in Groups of Rats Kept on a Synthetic Diet for One Hundred and Fifty Days* *

Daily Supplement	Animals	Fat Infiltration	Necrosis	Cirrhosis	Deposition of Ceroid
None.....	10	++	±	+	++
Cystine (50 mg.).....	20	+++	±	++	++
Choline (20 mg.).....	4	+	+	±	0
Cystine (50 mg.) and choline (20 mg.)....	5	+	0	0	0
Methionine (40 mg.).	4	++	0	0	0
Methionine (20 mg.).	1	++	0	+++	+++

* The synthetic diet (diet L-2) consisted of casein 10 per cent, lard 20 per cent, sucrose 64 per cent, salt mixture 4 per cent, cod liver oil 2 per cent and crystalline fractions of vitamin B.

The livers from animals that did not receive supplements revealed moderate fat infiltration and cirrhotic changes with considerable amounts of ceroid. Administration of cystine increased the fat infiltration and the cirrhotic changes without increasing the amount of ceroid. Administration of choline reduced the fat infiltration and the cirrhotic changes and prevented the formation of ceroid. Simultaneous administration of cystine and choline entirely prevented cirrhosis and the formation of ceroid, whereas administration of 20 mg. of methionine to 1 rat had no significant influence, while 40 mg. of methionine prevented cirrhosis and deposition of ceroid. No relationship between deposition of ceroid and necrotic and fatty changes was established.

In table 2 the average incidence of cirrhosis and of ceroid in the groups of rats on the different diets is given. In all of the different groups cystine enhanced the development of cirrhosis and the deposition of ceroid. Choline prevented the deposition of ceroid entirely and the development of cirrhosis to a marked degree, whereas simultaneous administration of cystine and choline prevented both changes almost entirely. Administration of p-dimethylaminoazobenzene enhanced the development of cirrhosis but almost completely prevented the deposition of ceroid. Rats that received p-dimethylaminoazobenzene showed deposition of ceroid only when they also received supplements of cystine.

The identical protective action of choline and butter yellow on the formation of ceroid is based probably on a common chemical denominator, i. e., on the presence of a labile methyl group in both chemical compounds. This assumption with the underlying observations is in accord with the findings of Jacobi and Baumann,[12] according to which p-dimethylaminoazobenzene, in analogy to choline, has a preventive effect on the bilateral cortical hemorrhagic necrosis of the kidneys which is produced in rats by a diet deficient in choline.

12. Jacobi, H. P., and Baumann, C. A.: Cancer Research **2**:175, 1942.

The influence of each of the basic dietary components is best evaluated if the rats with supplements, especially those with cystine, are also considered. Reduction of the casein (methionine) content of the diet increased ceroid and cirrhosis, as seen from the rats on diets L-2 and L-3 that received cystine. Substitution of butter for lard reduced the cirrhosis but not the ceroid. Hydrogenated cottonseed oil (crisco) had the same effect as lard. Cottonseed oil caused less deposition of ceroid, as seen from diet BY-2. Rice seemed to reduce the deposition of ceroid slightly (see animals on diet BY-4); otherwise, the type of carbohydrate administered (corn starch or sucrose) had no effect.

fluorescent microscope as well as by staining methods in visible light, but none was found in any of these livers. This study included many livers with cirrhosis, acute diffuse necrosis and hemochromatosis.

COMMENT

Microscopic examination for fluorescence indicates that the fluorescent material found in dietary nutritional cirrhosis of rats is identical with ceroid, which was first discovered by various staining reactions and chemical properties.[13] It differs from other fluorescent materials in the animal body by its stability against organic solvents, fixatives and light. The chemical nature of this somewhat inert material

TABLE 2.—*Average Incidence of Cirrhosis and Deposition of Ceroid in Rats on Synthetic Diets With and Without Supplements of Choline, Cystine and Butter Yellow*

	Constituents of Diet *								No Supplement			Cystine 50 Mg.			Choline 20 Mg.			Cystine (50 Mg.) and Choline (20 Mg.)		
Name of Diet	Casein	Rice	Sucrose	Corn Starch	Hydrogenated Cottonseed Oil	Lard	Butterfat	Cottonseed Oil	Rats	Cirrhosis	Ceroid	Rats	Cirrhosis	Ceroid	Rats	Cirrhosis	Ceroid	Rats	Cirrhosis	Ceroid
L-2	10	..	64	..	..	20	..	..	10	+	++	20	++	++	4	±	0	5	0	0
L-3	6	..	15	50	..	23	..	..	5	++	++	10	+++	+++						
Butter	6	..	15	50	..	..	23	..	3	0	++	4	+	++	..	..	..	3	0	0
Hydrogenated cottonseed oil	8	..	48	..	38	..	..	..	22	++	++	7	++	++	..	..	..	6	±	±
Rice	8	48	..	..	..	38	..	..	6	+	+	7	++	++	6	0	0	6	0	0
Diets Supplemented by 0.06 per Cent Butter Yellow (BY)																				
BY-2	6	..	22	50	..	..	..	16	..	..	..	6	+	+	..	..	..	6	0	0
BY-3	6	..	15	50	..	..	23	..	6	+	0	10	++	++	3	±	0	5	0	0
BY-4	6	65	..	..	..	23	..	..	13	++	0	14	+	+	11	+	0	11	0	0
BY-5	6	..	33	50	..	..	5	..	4	+++	0	4	++	++	3	+	0			
BY-6	6	..	15	50	..	23	..	..	11	+	±	23	++	++	7	+	0	8	0	0
BY-7	6	..	15	50	23	..	..	..	7	++	+	3	+++	++	..	..	..	2	++	0

* In all diets 4 per cent salt mixture, 2 per cent cod liver oil and fractions of vitamin B are included.

Curative Experiments.—One rat was first kept for one hundred and fifty days on the hydrogenated cottonseed oil diet. Ascites with hypoproteinemia (serum protein level 2.5 per cent) developed. For the following two hundred days a supplement of 50 mg. cystine and 20 mg. choline was given daily. The ascites disappeared, and the blood proteins rose to 6.2 Gm. per hundred cubic centimeters of serum. In the liver of the animal when killed the picture of arrested cirrhosis was encountered. In the sharply demarcated connective tissue strands macrophages containing dark brown fluorescent ceroid were seen, which appeared strongly colored in visible light.

Examination of Human Livers.—More than two hundred normal and pathologic human livers were examined for ceroid under the

is unknown. György and Goldblatt [1] suggested that it is a lipid-protein combination and Lillie and co-workers [2] and White and Edwards [6] considered it a conjugated lipoid substance derived from disintegrated hepatic cells.

We found the formation of the fluorescent material not related to hepatic necrosis, with or without hemorrhage, and also not associated with destruction of blood, as seen in hemorrhages. The concomitant occurrence of the deposition of ceroid and cirrhosis raises the question of a causative relation between them. Both are enhanced by low casein (methionine) diets and by cystine. The development of each is reduced by choline and nearly completely prevented by choline and cystine, as well as by

13. György and Goldblatt.[1] Lillie and others.[2] Edwards and White.[5]

methionine. György and Goldblatt [1] have assumed that choline and cystine combined act like methionine, choline providing the necessary labile methyl groups (du Vigneaud and associates [14]). Whipple and his co-workers [15] showed also that methionine protects against hepatic damage produced by chloroform in hypoproteinemic animals. In our experience the protection by choline and methionine against the deposition of ceroid is far more complete than in the experiments of White and Edwards.[6]

However, the negative findings for ceroid in rats that received p-dimethylaminoazobenzene, in many of which cirrhosis and hepatic tumors developed, show that even in the rat cirrhosis and deposition of ceroid are not necessarily combined. The liver in which cirrhosis is associated with marked bile duct proliferation and cellular infiltration as seen in these rats is almost free of ceroid, whereas that in which cirrhosis is associated with fat infiltration is rich in it. That brings up the question whether the formation of ceroid is related to the fatty changes.

The pictures seen by us suggest that ceroid fluorescence appears first in certain fat droplets of the liver cells. Soon afterward neighboring Kupffer cells contain it. Ceroid is then taken up by phagocytic cells, the foam cells. The latter may remain within the parenchyma of the hepatic lobules but usually accumulate in the portal spaces or central zones or in strands of dense connective tissue connecting them. In recent cirrhosis the ceroid deposits are not sharply demarcated. In older forms they are restricted to the connective tissue strands or to isolated accumulations of foam cells. The ceroid is apparently concentrated during this development, as judged from the change of the fluorescence from bright yellow to golden brown and the darkening of the visible color; the color finally becomes so strong as to prevent fluorescence entirely. It thus appears that ceroid is a fat pigment which undergoes concentration and remains in histiocytic macrophages when the fat disappears or healing occurs (Lillie and coworkers [2]).

Almost all of the rats of our series were kept continuously on the deficient diet. Nevertheless, the possibility exists that they later overcame the stage of fat infiltration which had developed in the first experimental period, as changes due to choline deficiency may retrogress, although the animals are still kept on the choline-deficient diet.[16] The presence of ceroid in connective tissue of fat-free livers may be a sign of a previous period of fat infiltration.

The assumption that ceroid is a fat pigment remaining and concentrated after the disappearance of fat is supported by the morphologic similarity to other pigments with brown fluorescence which have a similar relation to lipids. Such pigments are found where the sterols are formed in the body. Under the fluorescence microscope ceroid looks similar to the brown "wear and tear" pigment which remains after the disappearance of fats from the theca lutein cells of the human atretic follicle in the ovary.[17] This brown fluorescent material is similarly inert, shows a green color in visible light and stains with sudan stain even in paraffin sections from which ordinary fat and lipoid substances have been removed. It also does not give the reaction for iron and is not derived from blood pigment. Here also concentration with increase of visible color and fluorescence is observed, while the fat disappears. A similar pigment has been reported in the regressing corpus luteum of Macaca mulatta.[18] Brown fluorescent material is also seen in the Leydig cells of the testicle and the reticular layer of the adrenal gland. In the latter the brown "wear and tear" pigment is considered a remnant after the fat and the hormones present in the follicular layer have disappeared.

Ceroid may thus be considered as a concentrated fat pigment of the character of the "wear and tear" pigment formed in the pathologic fat turnover which is related to the development of certain forms of experimental nutritional cirrhosis of rats. That would not exclude it from being chemically a lipid conjugated with a protein.

SUMMARY

A golden brown fluorescent material is seen in liver from rats with experimental nutritional cirrhosis produced by a diet low in casein (methionine). Its deposition is enhanced by administration of cystine and prevented by administration of choline alone, of cystine and choline combined and of p-dimethylaminoazobenzene. The fluorescent material is apparently identical with ceroid, which was described before and identified by other means. It is now assumed that it develops from cells containing fat and is related to the fatty stages in the development of cir-

14. du Vigneaud, V.; Chandler, J. P.; Moyer, A. W., and Keppel, D. M.: J. Biol. Chem. **131**:57, 1939.

15. Miller, L. L.; Ross, J. F., and Whipple, G. H.: Am. J. M. Sc. **6**:200, 1940. Miller, L. L., and Whipple, G. H.: J. Exper. Med. **76**:421, 1942.

16. Griffith, W. H.: J. Nutrition **22**:239, 1941.

17. Ragins, A. B., and Popper, H.: Arch. Path. **34**:647, 1942. Popper.[11]

18. Rossman, J.: Anat. Rec. (supp.) **79**:53, 1941.

rhosis. Ceroid is a relatively inert material that may be considered as a pigment which is concentrated in the liver cells while the fat disappears and is then taken up by mesenchymal foam cells. In the latter it remains as a permanent morphologic sign of the disturbed fat metabolism.

NOTE.—After submission of this article, two papers which had just appeared came to our attention.[19] The authors report that ceroid imparts a greenish yellow fluorescence which changes slowly to yellowish white. They give a thorough description of the staining reactions, the other characteristics and the distribution of ceroid. Some facts mentioned by them appear also in our description. They show that ceroid does not belong to the groups of hemofuscin, anthocyanin, sterols, carotenoids, porphyrins or hemoglobin derivatives and consider an origin "from what in earlier stages of the process appears to be neutral fat." On the basis of staining reactions, ceroid is thought to be similar to the lipoid "pigment" of pneumonia due to aspiration of cod liver oil and to substances produced by injection into animals, and by oxidation in vitro, of oils containing unsaturated long chain fatty acids.

19. Endicott, K. M.: Similarity of the Acid-Fast Pigment Ceroid and Oxidized Unsaturated Fat, Arch. Path. **37**:49, 1944. Endicott, K. M., and Lillie, R. D.: Ceroid, the Pigment of Dietary Cirrhosis of Rats: Its Characteristics and Its Differentiation from Hemofuscin, Am. J. Path. **20**:149, 1944.

CALCIFIED EPITHELIOMA

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Since the latter part of the last century there have been occasional reports of a peculiar type of tumor of the skin and subcutaneous tissue, most commonly called calcified epithelioma. Tumors of this type are clinically noncancerous, encapsulated, and show a large amount of epithelial degeneration and usually focal calcification. Less frequently bone formation is seen. They are not described in the usual texts of pathology, although Kaufmann [1] discussed them in brief. Ewing [2] merely mentioned "petrifying epithelioma" in a discussion of acanthoma.

Malherbe and Chenantais [3] in 1880 were the first to describe this type of tumor as calcifying epithelioma of sebaceous glands. Most of the reports on such tumors since then have appeared in the French and German literature. The first comprehensive report in the English literature is that of Ch'in,[4] who in 1933 reported on 10 tumors with this diagnosis gathered from over 22,000 surgical specimens covering a period of fifteen years. He made an extensive review of the literature from which he collected an additional 116. Sutton and Sutton [5] described another in 1935. Coté [6] reported 12 in 1936. We are reporting on 1 probable and 11 definite tumors of this type gathered from a series of 24,185 surgical specimens received over a period of fifteen years.

GENERAL DESCRIPTION

Clinically, most of our tumors of this type were thought to be cysts. They were slow growing, and their recorded duration prior to removal ranged from a few days to ten years, with an average of about three years. Four tumors were on the head, 4 on the neck and 1 each on the upper arm, the forearm and the buttock. The location of 1 was not recorded. Patient 4 had 1 tumor on the neck and 1 on the left upper arm; a third tumor in the right axilla was not submitted for examination. Four tumors were in 4 females. Four patients were under 20 and 2 were over 60. One tumor was first noticed at 6 months and 1 at 10 months of age. There have been no known recurrences, but our follow-up studies are incomplete.

Grossly, the tumors were usually adherent to the overlying intact skin but were freely movable over the deeper tissues. Six specimens contained bone, and 5 of these were hard enough to require decalcification before microscopic sections could be prepared. Other specimens were soft and cheesy and often had a characteristic compressed-sawdust-like or sandy-appearing cut surface.

Microscopically, the tumors were composed of rounded, lobulated or irregularly shaped masses and strands of epithelium supported by connective tissue trabeculae that were continuous with an outer condensed connective tissue layer or capsule. Small islets of epithelium were seen also in the capsule and less frequently in the pericapsular tissue. Most of the epithelial cells were degenerated. In 4 specimens no viable or well preserved epithelium was clearly identified (cases 4, 6, 7 and 10); in a fifth (case 11) most of the epithelium was viable, and in the remainder there were isolated areas of surviving epithelium, seen chiefly in the outer portion of the tumor, where they occurred as separate masses or more commonly as bands or rings bordering the degenerated epithelium or in continuity with it.

The viable cells were generally small polygonal and coherent, and had scanty, deeply basophilic cytoplasm with indefinite borders. The nuclei were round to oval and had fine to coarse chromatin. Mitoses varied from none to numerous. These cells resembled closely those of basal cell epithelioma but were generally smaller and more uniform in appearance. Here and there in small

From the Division of Pathology, National Institute of Health.

1. Kaufmann, E.: Pathology for Students and Practitioners, translated by S. P. Reimann, Philadelphia, P. Blakiston's Son & Co., 1929, vol. 3, p. 2197.
2. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940, p. 888.
3. Malherbe, A., and Chenantais, J.: Bull. et mém. Soc. anat. de Paris **15**:169, 1880.
4. Ch'in, K. Y.: Am. J. Path. **9**:497, 1933.
5. Sutton, R. L., and Sutton, R. L., Jr.: Arch. Dermat. & Syph. **31**:48, 1935.
6. Coté, F. H.: J. Path. & Bact. **43**:575, 1936.

patches the cells were larger, less deeply basophilic and more angular or flattened, and often showed intercellular bridges. In general, they resembled prickle cells and occasionally showed keratohyaline granules, keratinization and pearl formation. For brevity we shall henceforth designate these two cell types as basal and squamous respectively.

Epithelial degeneration varied in character. In most areas the degenerated cells were similar to the viable cells in size, shape and arrangement, but their outlines were more distinct, their cyto-

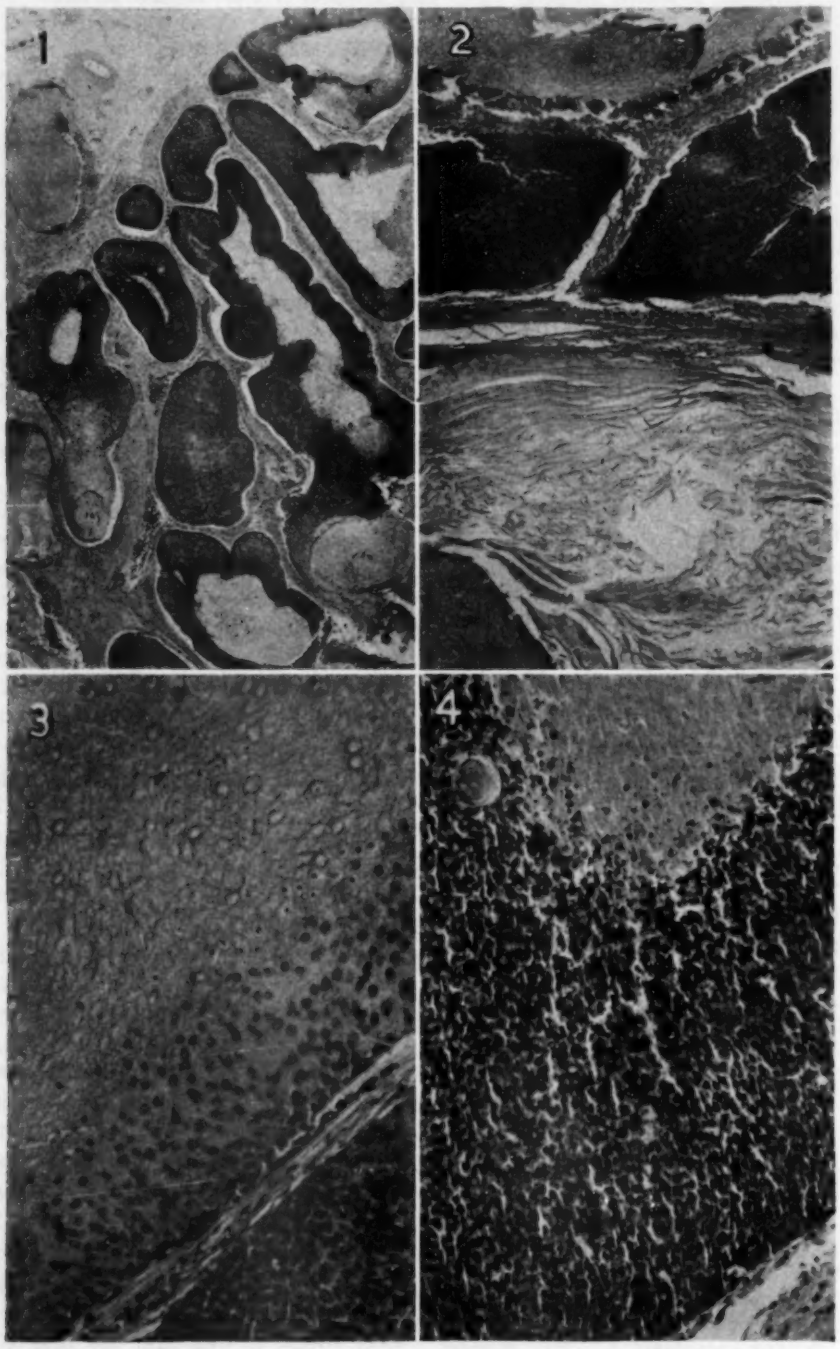

Fig. 1 (case 8).—Low power showing general structure. × 20.

Fig. 2 (case 8).—Portion showing various epithelial changes. × 85.

Fig. 3 (case 8).—Area showing dyskeratosis. Note pyknosis in the transition zone. × 160.

Fig. 4 (case 8).—Portion of an epithelial ring showing change to amorphous granular debris. Note the small keratin body. × 160.

plasm was deeply eosinophilic, and the relatively large nuclei were less deeply eosinophilic or appeared as round vacuoles, often containing an eosinophilic nucleolus. To avoid descriptive repetition, we shall designate epithelium exhibiting such changes as dyskeratotic. In 6 specimens (cases 4, 5, 6, 7 and 10) the degeneration appeared to be entirely of this type. Occasionally these dyskeratotic cells were slightly separated, suggesting dissolution of the intercellular cement substance in the process of fixation.

Occasional bands of viable epithelium and some dyskeratotic masses showed one or more small rounded bodies. Some of these bodies were hyalinized, some were composed of dyskeratotic epithelium, some were concentrically laminated and essentially similar in structure to epithelial pearls of acanthoma, and many showed intermediate changes. Less frequently, bands of viable epithelium showed single and small groups of large round keratinizing cells with eosinophilic cytoplasm. The nuclei of such cells were often vesicular and well preserved but sometimes were pyknotic or resembled those of the dyskeratotic epithelial cells. Most tumors also showed occasional masses of keratin squames and some laminated keratin structures, sometimes merging into dyskeratotic epithelium. These structures were usually associated with patches of squamous epithelium from which they were sometimes separated by a thin stratum granulosum with keratohyaline granules and sometimes also by a few flattened layers of parakeratin. The squamous patch might be marginated peripherally by basal cells, thereby duplicating the layers of the epidermis.

In some tumors, especially in the interior of the specimens, some epithelial masses were replaced at least centrally by areas of necrotic amorphous debris, in which there could occasionally be seen some keratin or hyaline bodies or islets of dyskeratotic epithelium. These necrotic areas might be marginated by either viable or dyskeratotic epithelium. In most areas the change from viable to dyskeratotic or necrotic epithelium was abrupt, but focally there might be a few intervening layers of cells with lightly eosinophilic cytoplasm and pyknotic nuclei. In such areas the dyskeratotic epithelium always bulged slightly away from the slide, making it difficult to get satisfactory photomicrographs. There were also occasional areas of karyorrhectic necrosis, occurring chiefly as a narrow zone between viable epithelium and some of the areas of amorphous debris.

All of our tumors that were not decalcified prior to sectioning showed some evidence of focal calcification, usually in the form of a fine basophilic powdering of the dyskeratotic epithelium or occasionally in that of small plaques. In such areas the nuclei and the nucleoli of the dyskeratotic cells were often lightly basophilic with Giemsa's stain but poorly stained with hematoxylin. Less commonly calcification was seen in the stroma, usually in the form of incrustations of the fibers, which appeared as deeply basophilic lines or in the form of solid irregular masses or small granules. The presence of calcium was confirmed in most cases by Kossa's silver nitrate method.

Six of our tumors showed bone formation, which varied from a few spicules to extensive ossification with marrow-like spaces, giving the tumor the appearance of cancellous bone. The bone spicules showed well differentiated bone corpuscles and bone matrix. They had the same general contour as, and were closely applied to, the surface of the dyskeratotic and often marginally calcified epithelial masses, occurring either as a thin fringe or as larger marginating areas partially or completely replacing the epithelium. Sometimes slender tongues of bone matrix were seen extending between or completely investing the bordering dyskeratotic cells. The larger masses of bone often showed small inclusions of dyskeratotic epithelium and cavities containing capillaries and loose fibrous tissue. In 4 tumors (cases 4a, 5, 7 and 10) the area of bone was only slightly less than that of the epithelium, but the proportions in different areas varied widely. The stroma between osseous cancelli was composed largely of loose fibrous tissue, often resembling fatty bone marrow and showing few to many adult fat cells or, in case 5, masses of large foam cells suggesting embryonic fat. The stroma in other areas varied considerably in amount and density, was generally moderately cellular, occasionally was focally hyalinized (cases 6 and 11) or basophilic (case 11) and often showed some extravasated red blood cells and occasional hemosiderin-laden phagocytes, and an irregular infiltration by lymphocytes, sometimes large mononuclear cells and occasionally a few neutrophils and plasma cells. Multinucleated foreign body giant cells, many of them containing fragmented dyskeratotic material, were commonly seen especially bordering the dyskeratotic and necrotic epithelial masses. The latter often showed apparent organization with partial replacement by a cellular connective tissue ingrowth containing many giant cells and large mononuclear cells.

The capsules of some of the tumors (cases 4a, 4b and 11) could not be clearly identified and differentiated from the stroma and that of 1 (case 10) was thin and incomplete. When pericapsular structures were present and could be identified

in the sections, the tumor was found to lie in the deeper derma and subcutaneous tissue, which were focally slightly infiltrated, chiefly by lymphocytes. The epidermis when present was generally thin and atrophic, the interpapillary rete largely suppressed.

Fat stains, sudan IV in 60 per cent isopropanol being used,[7] were made only on material from cases 8, 9 and 11. The lipoid substance was largely isotropic and occurred as minute droplets in the necrotic epithelium and in large mononuclear cells. A lesser amount was occasionally seen in some small areas of dyskeratotic epithelium.

years when it suddenly seemed "to have burst inside and spread out." In this area there then developed the present smaller firm mass which grew slowly and was intermittently painful for several years before removal. The tumor in case 11 is reported as probably calcified epithelioma largely because no dyskeratotic epithelium was clearly identified. The viable cells were basal in type but were frequently larger and paler than those in the preceding cases and focally varied considerably in size and shape. There were many scattered single and small groups of the keratinizing cells previously described, but no definite squamous cells or keratin bodies. Some

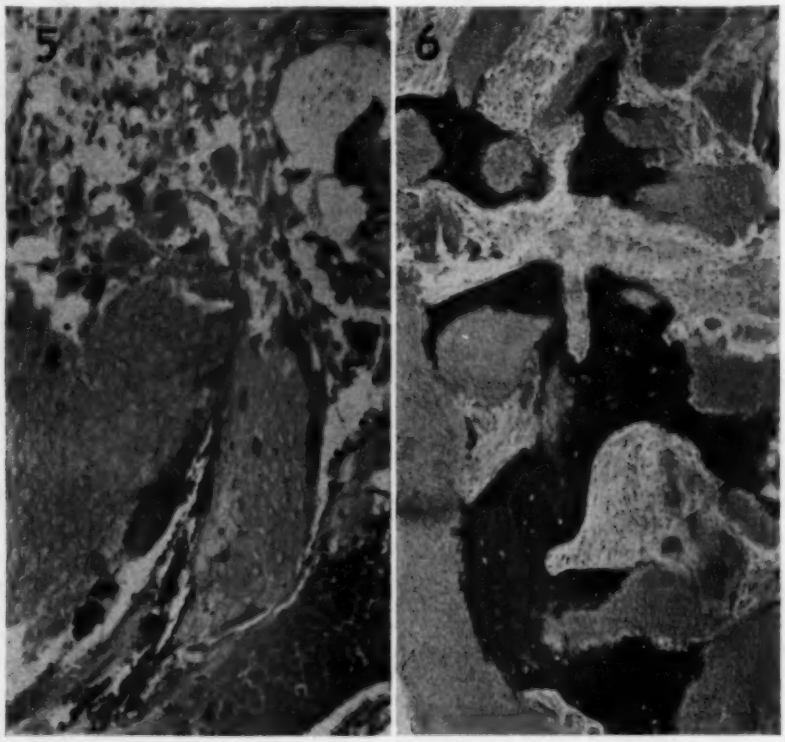

Fig. 5 (case 8).—Area showing a foreign body reaction and calcium deposits in the stroma. × 160.
Fig. 6 (case 5).—Area showing bone formation and dyskeratotic epithelium. Van Gieson stain. × 85.

Most of the case variations are recorded in the accompanying table. A few additional remarks are in order.

Two specimens (cases 2 and 3) showed a very small amount of brown pigment resembling melanin in a few laminas of parakeratin. One tumor (case 9) showed a moderate amount of similar pigment in some dyskeratotic masses. The presence of such pigment is not mentioned by Ch'in in his review of the literature. In case 10 a mass was first noted in front of the right ear at 6 months. This grew in prominence for about five

cell masses contained one or more round or irregularly shaped spaces of variable size. Most spaces were empty, but some contained keratin flakes and many were marginated by a layer of cuboidal or polyhedral cells with oxyphilic cytoplasm often containing keratohyaline granules. Some of these cells closely resembled or were identical with the keratinizing cells. Some of the larger spaces showed simple or complexly papillary infoldings.

COMMENT

Our finding of 11 specimens of calcified epithelioma among 24,185 consecutive surgical specimens compares roughly with Ch'in's 10

7. Lillie, R. D., and Ashburn, L. L.: Arch. Path. 36:432, 1943.

gathered from over 22,000 surgical specimens at the Union Medical College in Peiping, China. During this period, from 1928 to 1943, the pathologic laboratory of the National Institute of Health has had about 200 specimens of acanthoma of the skin and a slightly larger number of basal cell carcinoma. The relative paucity of reports in the American literature suggests that many tumors of this type are not differentiated or recorded. Doubtless, too, many are diagnosed clinically as sebaceous cysts and are not examined.

their form, shape and coherence, it appears that such commonly used terms as "necrosis" or "eosinophilic degeneration" inadequately describe this alteration. We believe that the pathologic process is basically similar to the change seen in epithelial cells in certain cutaneous diseases and designated as dyskeratosis. The eosinophilic or hyaline appearance of the cell cytoplasm, the occasional association and merging of such cells with laminated keratin structures and small round keratin bodies, and the persistence of cell form for considerable intervals after death appear to

Principal Features of Eleven Cases of Calcified Epithelioma

	Clinical Features							Microscopic Features: Epithelium									
Case	Age of Patient	Race	Sex	Location of Tumor	Greatest Diameter, Mm.	Recorded Duration	Clinical Diagnosis	Basal Cells	Squamous Cells	Mitoses	Dyskeratosis	Necrosis	Laminated Keratin	Keratin Bodies	Calcification	Ossification	Foreign Body Giant Cells
1	66	White	M	Near eye (L)	16	5½ mo.	Malignant tumor	+	±	±	++++	+	+	+	+	0	+++
2	51	White	F	Neck (R)	12*	1 yr.	Sebaceous cyst	+	±	±	++++	+	+	+	+	0	+++
3	61	White	M	Forearm (R)	20*	3 yr.	Calcified sebaceous cyst	+	±	+	++++	+	+	+	D	0	++
4	28	White	M	(a) Upper arm (L)	30	Many years	Calcified retention cyst	0	0	0	++++	0	0	±	D	++++	+
				(b) Neck	27*	Many years	?	±	±	0	++++	0	±	+	+++	0	+++
5	19	White	M	Neck (L)	25	1 yr.	?	±	0	0	++++	0	0	±	D	+++	+++
6	50	White	M	Buttock (R)	25	1 yr.	Saponified lipoma	0	0	0	++++	0	±	+	++	±	+
7	?	?	?	?	8*	?	?	0	0	0	++++	0	0	±	D?	++++	+
8	9	Mixed Indian	F	Neck (nape, L)	45	2 yr.	Benign cyst	++	+	++	++	+++	+	++	++**	+	+++
9	1½	Japanese	F	Upper eyelid (R)	14	7 mo.	Granuloma	++	+	++	+++	+	+	++	++	0	+++
10	10	White	F	Temple (R)	15	9½ yr.	Sebaceous cyst	0	0	0	++++	0	0	±	D	++++	++
11	65	Indian	M	Scalp	50	3 or 4 yr.	Sebaceous cyst	++++	0	+++	0	++	0	0	+	0	±

0 = none; ± = very small or equivalent; + = small; ++ = moderate; +++ = large; ++++ = very large; * = diameter of microscopic preparation; ** = in portion not decalcified; D = decalcified.

The clinical findings in our cases are comparable with those recorded in the literature. Many authors have recorded a preponderance of females among the patients. The finding of 4 tumors in our series in females, considering that adult males account for by far the greater portion of pathologic material at this laboratory, suggests an extraordinary preponderance in females.

The histologic picture of these tumors is characteristic. We have found that the most constant and distinguishing feature is the peculiar dyskeratosis. There is no doubt that this epithelium is nonviable. Its presence in all our definite cases irrespective of the recorded duration suggests that death of the cells antedated removal of the tumors by many months or years. When one remembers that the epithelial cells have retained

support this view. Six of our specimens show bone formation, which is a much higher proportion than is usually recorded. Thus Coté found none among his 12 tumors. This suggests that many soft tumors were not submitted for examination.

The origin of calcified epithelioma is not clear. It has been regarded variously as endothelioma, atheroma, basal cell carcinoma, a derivative of an epithelial rest, a result of traumatic epithelial inclusions, cholesteatoma and dermoid and epidermoid cyst. Malherbe suggested that the lesion results from abnormal proliferation of the lining wall of a sebaceous cyst. Kaufmann stated that the origin may be in preformed glands as well as in deep lying (dystopic) epithelial cells, probably misplaced anlage material. Coté expressed the

belief that this is a lesion sui generis, derived possibly from the anlage of a sebaceous gland, in which the final growth occupies an intermediate position between the ordinary epidermoid cyst and the basal type of carcinoma and exhibits some of the characters of both. Some of our tumors showed occasional bands of viable epithelium focally differentiating into laminated keratin with an arrangement of the various layers similar to that of the epidermis. These bands were seen in different portions of the tumors and occasionally immediately beneath the capsule and suggested an origin similar to that of an epidermoid cyst, a view held by Virchow, Joannevics and Sternberg. The origin of such cysts, according to Warvi and Gates,[8] may be the epidermis, sebaceous ducts and hair follicles. We do not particularly favor the first two as possible sites of origin because our tumors appeared to lie in the deeper corium and subcutaneous tissue and in none did we find any recognizable cells of a sebaceous gland. On the other hand, in several of our tumors, especially that in case11, some of the viable cells closely resembled those in the center of hair bulbs. The presence of pigment resembling melanin in parakeratin and dyskeratotic masses in 3 tumors (cases 2, 3 and 9) suggests an analogy with the pigment found in the horny epithelial cells of hair shafts. Hence, we are inclined to believe that at least some of these tumors have an origin in structures similar to hair follicles or from these.

There have been objections to the term "calcified epithelioma." Some have objected to "calcified" since calcification is considered to be secondary to the degeneration and may be minimal or absent. Others have objected to "epithelioma" on the basis that these tumors are almost always at least clinically noncancerous and that term is generally used in connection with carcinoma. Another inadequacy is the lack of a descriptive term for the peculiar dyskeratosis. However, since the origin of these tumors is not clearly understood at present and to avoid confusion, we have retained the commonly used term "calcified epithelioma."

In the treatment the simple surgical removal of the tumor usually suffices. However, the prognosis must be somewhat guarded since occasional recurrences have been reported. Coté [6] reported two recurrences in his 12 cases. Gromiko [9] reported two apparent recurrences of calcified epithelioma on the arm. The tumor was considered cancerous both clinically and pathologically, and the arm was amputated. The follow-up in our series is incomplete, but in those cases in which information is available, there have been no recurrences.

SUMMARY

The 11 specimens and 1 probable specimen of calcified epithelioma described in this article were gathered from 24,185 consecutive surgical specimens. One patient had 2 tumors of this type. Most of the tumors were thought to be cysts. Four were in 4 females. The youngest patient was 17 months and the oldest 66 years of age. There were no known recurrences.

Microscopically, the tumors were composed of masses of epithelium supported by connective tissue trabeculae that were continuous with a capsule. The most characteristic feature appeared to be eosinophilic degeneration of the epithelium, which we believe is a form of dyskeratosis. In 4 tumors all the epithelium was of this type. In the others isolated areas of surviving epithelium occurred as separate masses or as bands bordering the degenerated epithelium. All 7 tumors not previously decalcified showed some calcification. Six showed bone formation, an unusually high percentage. Three contained a small amount of pigment resembling melanin.

The origin of such tumors is not clear. However, we believe that some may have an origin from or similar to that of hair follicles. The commonly used term "calcified epithelioma" is unsatisfactory but is retained to avoid confusion.

8. Warvi, W. N., and Gates, O.: Am. J. Path. **19**: 765, 1943.

9. Gromiko, N.: Virchows Arch. f. path Anat. **265**:103, 1927.

LYMPHADENITIS OF SECONDARY SYPHILIS

ITS RESEMBLANCE TO GIANT FOLLICULAR LYMPHADENOPATHY

NEWTON EVANS, M.D.
LOS ANGELES

Attention is directed to a striking histologic similarity existing between the lymphadenitis of secondary syphilis and so-called giant follicular lymphadenopathy as illustrated in the following cases.

CASE 1

D. C., a Negro man aged 24, married, was admitted to the Los Angeles County Hospital Aug. 20, 1943 and remained until September 11. He stated that he was in good health until two weeks before admission, when nausea began, followed by vomiting. Four days later pain developed in the left lower quadrant of the abdomen. It radiated to the upper part of the epigastrium and was relieved by vomiting. The patient gave a history of having had gonorrhea two years before and a second attack one month before entry. He also stated that one month before he was admitted the penis was "burned" and that a "blister" had been present thereafter. He further stated that a Wassermann test made one month before admission was negative.

There was a nonulcerated lesion on the foreskin which was described as a 1.5 cm. size round plaque, raised above the surface of the skin and in part firmly indurated. The only abnormalities noted in addition were multiple hard superficial lymph nodes distributed in the submaxillary, cervical, axillary, inguinal, popliteal and epitrochlear regions. Also a small plaque, 3 by 6 mm., was seen at the anterior border of one tonsil. Tissue removed from the penile lesion was stained and examined for spirochetes, and none were found.

An initial diagnosis was not made, but these possibilities were considered: (1) infectious mononucleosis; (2) secondary syphilis; (3) lymphoblastoma, possibly Hodgkin's disease.

The hematologic findings were not definitely abnormal. A test with heterophil antigen was negative. The Wassermann and Kahn tests were strongly positive on three occasions while the patient was in the hospital, the first on the day of entry.

On September 3 a large node was removed from the epitrochlear region for examination. Sections were stained with hematoxylin and eosin. I reported: "Lymphoma—probably malignant. There is a marked resemblance to follicular lymphoma." At that time a section stained with silver stain for spirochetes was requested, and examination of this revealed numerous spirochetes having the typical morphologic characteristics of Treponema pallidum. The organisms were found in considerable numbers and were almost entirely confined to the walls and the immediate vicinity of the hyperplastic blood capillaries and other minute blood vessels.

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CASE 2

F. M., a Mexican man aged 24, was admitted July 29 and left Nov. 8, 1943. He complained of multiple pains of the ankle joints, knees, one wrist and elbows and of cough with sputum for one month.

Numerous clinical and laboratory tests were made, all of which gave negative results except for positive Wassermann reactions on July 31 and August 20. The list of negative laboratory tests included blood cultures, a complement fixation test for gonococcus, the Widal test, an agglutination test for Brucella, a test with heterophil antigen, an intracutaneous coccidioidin test, staining of sputum for acid-fast bacilli and a blood count. No superficial adenopathy was noted on admission. The temperature, as shown by the record, was practically normal throughout the patient's three months' stay. The blood count on admission revealed 4,000,000 red cells with a hemoglobin content 76 per cent of normal; on October 18 the blood was essentially normal.

Numerous examinations of the patient were recorded, and various diagnoses were suggested, but no consensus as to the clinical diagnosis could be arrived at.

October 13 enlargement of cervical lymph nodes was noted, associated with sore throat and painful swallowing.

On October 18 several enlarged cervical lymph nodes, both posterior and anterior, were noted, as well as small hard axillary nodes and a few hard inguinal nodes. Also an eruption of the skin over the back and the arms was reported, described as "diffuse, blotchy, scaling."

On October 26 the dermatologic consultant considered the cutaneous lesion to be a "follicular syphilid." The opinion had been previously voiced that notwithstanding the positive Wassermann reaction the patient probably did not have syphilis and that this reaction might be a "false positive reaction." The absence of any primary syphilitic sore and the patient's denial of infection apparently contributed to this conclusion.

October 29 an enlarged cervical lymph node was removed for examination. It was rounded and 1.7 cm. in size. Sections were examined by me and reported as showing the microscopic characteristics of giant follicular lymphadenopathy, and a provisional diagnosis of this condition was made. However, the possibility of syphilitic lymphadenitis was entertained, and a stain for T. pallidum was made. Numerous spirochetes with characteristic appearance were demonstrated. These spirochetes were practically confined to the walls and immediate vicinity of the hyperplastic capillary blood vessels. The diagnosis of "syphilitic lymphadenitis probably associated with a secondary stage of syphilis" was then made.

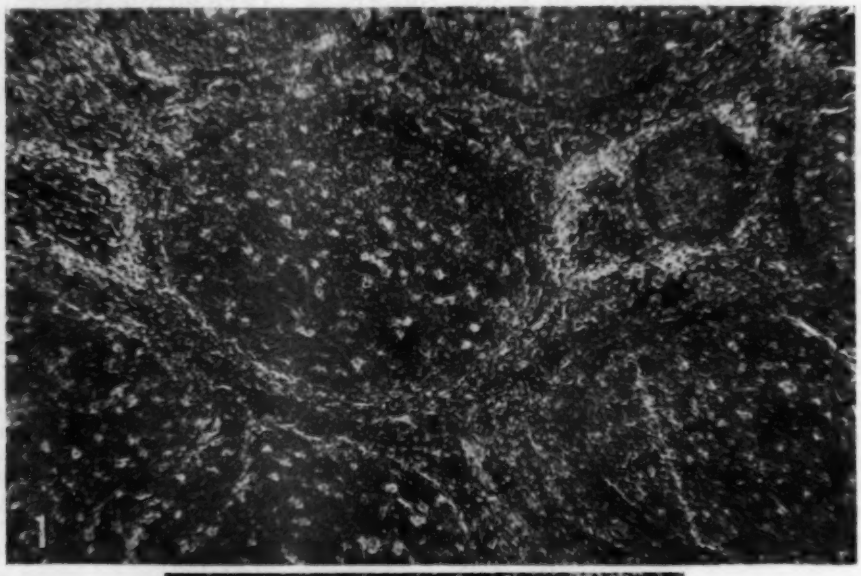
COMMENT

One of the most difficult routine diagnostic problems of the pathologist is presented in the study of lymph nodes removed for histologic examination. One characteristic group of lesions of these is that of metastatic cancers, which are

usually recognized as such with no great difficulty.

A second group includes the various types of specific infectious granulomatous lymphadenitis, such as tuberculosis, late syphilis, coccidioidal granuloma, histoplasmosis, Boeck's sarcoid and lymphogranuloma venereum. These all present fairly well recognized characteristics.

A third is the acute or subacute nonspecific lymphadenitis group, such as is illustrated by the changes seen in the regional lymph nodes in connection with inflammatory diseases of pyogenic orgin; it might include the lymphadenitis of the regional nodes in primary syphilis. Accurate histologic descriptions of these changes seem difficult to find in the literature. The



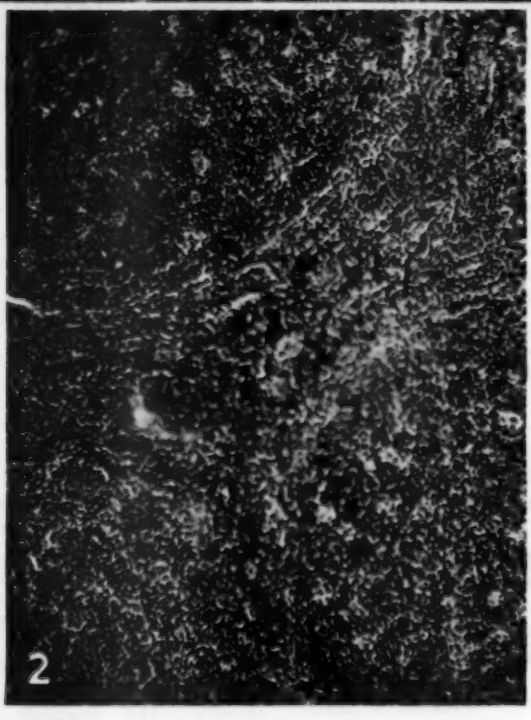


Fig. 1.—Low magnification of lymph node in case 2, showing the increase in number and in size of the follicles at the periphery and in the depth of the node; × 60.

Fig. 2.—Higher magnification of the node in figure 1, showing the enlarged follicles composed of lymphoblasts and large phagocytic reticulum cells, as well as the compressed interfollicular structures; × 150.

changes, however, usually include hyperplasia of the follicles.

Lastly, there is the group which makes the most difficulty, the true cancerous lymphadenopathies. Many attempts at an acceptable classification of these lesions have been made, based largely on the cytologic types which predominate. Gall and Mallory [1] recently submitted a classification which seems essentially complete and fairly satisfactory. It is unnecessary to enumerate their seven cytologic types. However, they include, as other workers in this field rather

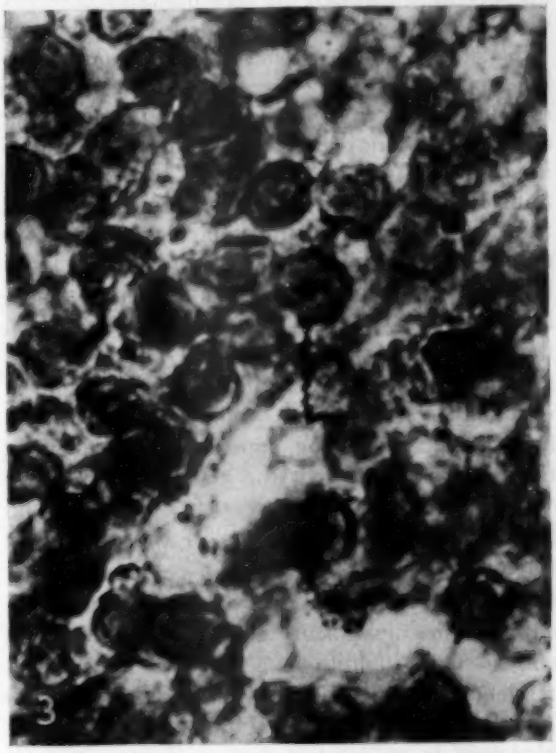


Fig. 3.—A single spirochete (T. pallidum) in close relation to a capillary blood vessel in the node shown in figures 1 and 2; × 1,200.

consistently do, the characteristic lesion (called by a rather confusing variety of names [2]). When first studied by American observers this lesion, the follicular lymphoma, was regarded as noncancerous. However, when it came to be investigated more intensively and the progress of the patients observed, the fact became evident that clinically it is a cancerous lesion. Practically all of the patients succumbed after the disease had run an average course of four and eight-tenths years. One of the most concise but essentially complete discussions of the disease process from the point of view of the pathologist is that of Baggenstoss and Heck.[3]

Changes in Lymph Nodes in Secondary Syphilis.—It is generally accepted that in a large proportion of the patients newly acquired syphilitic infection follows a clinical course consisting of more or less stereotyped stages with characteristic symptoms and pathologic changes, usually denominated primary, secondary and tertiary syphilis. The first stage includes the first few weeks in which the initial chancre is followed by enlargement of the regional lymph nodes. This is followed after a period of weeks or months by the so-called second stage with characteristic clinical symptoms and with lesions of the mucous membranes and the skin. This stage is usually characterized by widespread enlargement of the lymph nodes of the superficial groups. It seems reasonable to adopt the concept that the bodily changes are essentially similar in the primary and the secondary stage and are brought about by the invasion of the body tissues by T. pallidum, the two stages simply constituting the steps in the complete inundation of the body by the organisms. The tertiary stage, on the other hand, may be regarded as a delayed residual reaction of certain membranes and viscera to the organisms, which are harbored in the body in much smaller numbers in this stage than in the blood stream and the affected tissues in the earlier periods. In other words, it seems more logical from the pathologic point of view to consider syphilitic infection in two stages instead of three—early syphilis, including the first and second stages, and late syphilis, the period of the so-called tertiary lesions.

All authors in discussing the changes in the lymph nodes in syphilis refer to the usual finding of generalized enlargement of the superficial nodes in the secondary stage, but I have been unable to find satisfactory descriptions of the microscopic changes. Forbus [4] quoted Zurhelle's [5] detailed description of the regional lymphadenitis of the primary stage and then stated that "in the secondary stage the lymphoid

1. Gall, E. A., and Mallory, T. B.: Am. J. Path. **18**:381, 1942.

2. A list of the names includes: giant lymph follicle hyperplasia (Brill, Baehr and Rosenthal); malignant lymph follicle hyperplasia (Baehr and Rosenthal); follicular lymphoblastoma (Baehr); giant follicular lymphadenopathy (Symmers); follicular lymphadenopathy (Symmers); follicular type of malignant lymphoma (Gall, Morrison and Scott); follicular lymphoblastoma (Anderson: Synopsis of Pathology): follicular lymphoma (Gall and Mallory [1]).

3. Baggenstoss, A. H., and Heck, F. J.: Am. J. M. Sc. **200**:17, 1940.

4. Forbus, W. D.: Reaction to Injury, Baltimore, Williams & Wilkins Company, 1943.

5. Zurhelle, E.: Deutsche med. Wchnschr. **47**:1122, 1921.

reaction is like that in the primary complex." The changes in the lymph nodes are described as in no sense specific but are like those in almost any chronic lymphadenitis, consisting of proliferation of the cells of the lymphoid cell cords and the "cortical reaction centers" (germinal centers) and of the cells lining the sinuses. The sinuses are filled with cells (lymphocytes, plasma cells and large macrophages). It is stated that in the "reaction centers" necrotizing changes develop, with appearance of cells with cytoplasmic inclusions, but that softening and suppuration are uncommon. Occasionally mild fibrosis occurs. Karsner [6] stated (with reference to the secondary stage) that "microscopically there is subacute or chronic hyperplasia. The secondary nodules (germinal centers) may be enlarged and active. The sinuses show a moderate endothelial hyperplasia and fibrosis is usually pronounced." Bell [7] made the apparently unique observation, accompanied by an illustration, that he had observed "miliary gummas" in lymph nodes in the secondary stage.

In none of the discussions reviewed has there been found any description of the appearance of greatly enlarged follicles and increased numbers of follicles in the nodes, such as might suggest a resemblance to the microscopic picture characterizing giant follicular lymphadenopathy.

In each of the 2 cases of enlargement of lymph nodes in secondary syphilis reported here, the marked resemblance of the microscopic pattern to that of giant follicular lymphadenopathy was noted, and a provisional microscopic diagnosis of follicular lymphadenopathy was made. However, in both instances the possibility of syphilitic lymphadenitis was entertained, and sections were prepared with silver stain for spirochetes, resulting in the demonstration of numerous spirochetes having the morphologic characteristics of T. pallidum.

The distribution of the organisms in the lymphoid tissue is a point of some interest. They are obviously confined almost wholly to the walls and the vicinity of the hyperplastic vascular channels; however, it is possible to find considerable numbers in the hyperplastic germinal centers. When found in these structures, however, it is evident that they are largely in the immediate vicinity of the vascular structures there present. Interestingly, Zurhelle [5] stated that the organisms are never found in the germinal centers (reaction centers).

6. Karsner, H. T.: Human Pathology, ed. 6, Philadelphia, J. B. Lippincott Company, 1942.

7. Bell, E. T.: Text-Book of Pathology, ed. 4, Philadelphia, Lea & Febiger, 1941.

Differentiation from Follicular Lymphoma.—The paper of Baggenstoss and Heck [3] includes a clearcut and convincing discussion of the points of histologic differentiation between the inflammatory hyperplasia of the secondary nodules (germinal centers) of lymph nodes and the changes in "follicular lymphoblastoma." This, of course, is the exact problem which one faces here. These authors presented in tabulated form the contrasting microscopic observations under two headings—the follicles and the interfollicular tissue. The chief features of the differentiation are briefly expressed in this quotation: "The most helpful histologic sign in distinguishing follicular lymphoblastoma from inflammatory hyperplasia was the greater numerical and dimensional increase in the follicles in the former condition." They also stated that an additional important differential feature is a tendency of the follicles to fuse with each other and added that the changes in the interfollicular areas give much aid in the differentiation. The latter include the features that in follicular lymphoblastoma the lymphoid cells are densely packed, the reticulum is condensed, the lymph sinuses are narrowed or blocked and proliferation of reticulum cells is slight, while in inflammatory hyperplastic nodes the opposite is noted—i. e., the cells are scattered, the reticulum is loosely arranged, the sinuses are open or dilated and there is marked proliferation of the phagocytic reticulum cells.

The description given by Gall and Mallory is entirely in agreement on these points, and emphasizes an additional feature, viz., a marked tendency for the follicles in tissue sections to "crack off" from the surrounding tissue. This obviously is an artefact, but in their opinion it is apparently of diagnostic importance.

In the present study a comparison of the sections of the lymph nodes in the 2 cases with the foregoing criteria as well as with sections from other nodes previously recognized as showing follicular lymphoblastoma reveals some similarities to lymphoblastoma, namely, the size, the number and the distribution of the follicles and the fusion of adjacent follicles. On the other hand, the changes in the tissue areas between the follicles reveal greater resemblance to those described in inflammatory hyperplasia, particularly in the points pertaining to the lymph sinuses and the proliferation of the phagocytic reticulum cells. In case 1 a marked tendency toward dilatation of the sinuses and proliferation of reticulum cells within them and toward loosely scattered distribution of the lymphocytes is evident. In case 2 the open sinuses are not

evident, but large areas of typical large phagocytic reticulum cells displacing the lymphocytes are present.

A striking incidental feature of the section in this second case is entitled to brief mention. A large proportion of the machrophages contain abundant brown pigment granules. This is evidently an instance of the so-called lipomelanic reticulosis of Pautrier and Woringer,[8] a pigmentation of the reticulum cells in enlarged nodes associated with certain eruptive lesions of the skin. In my own observation of this phenomenon this pigment has usually been present in dark-skinned subjects. The patient in case 2 was a Mexican. Such pigmented macrophages were not observed in the node from the first patient, who was a Negro but one in whom no accompanying lesions of the skin were recorded.

It is of interest that Baggenstoss and Heck [3] in discussing the problem of differentiation recognized the possibility of encountering enlargements of lymph nodes in which the differentiation is impracticable. To quote: "In occasional cases, in which a conclusion could not be reached even after careful study, it was found convenient to reserve the diagnosis and to use a non-committal term such as 'follicular hypertrophy' to describe the histologic appearance."

8. Pautrier, L. M., and Woringer, F.: Ann. de dermat. et syph. **8**:257, 1937.

SUMMARY AND CONCLUSIONS

In 2 cases illustrative of the histologic changes in the superficial lymph nodes in the secondary stage of syphilis the histologic changes bore such a marked resemblance to those of giant follicular lymphadenopathy, a cancerous lymphomatous disease, that there appears to be a possibility of mistaking syphilitic lymphadenitis for that disease.

T. pallidum was demonstrated in tissue sections stained with a silver stain for spirochetes.[9]

These cases illustrate the usefulness of microscopic sections in the recognition of syphilitic lesions and particularly the importance of employing a dependable stain for spirochetes in arriving at a conclusive diagnosis of syphilis in its early stages.

9. The method used in these cases is that of Krajian (Am. J. Syph., Gonor. & Ven. Dis. **23**:617, 1939; Histological Technic, St. Louis, C. V. Mosby Company, 1940, pp. 163 and 166).

SPINDLE AND GIANT CELL SARCOMA ARISING FROM UNIDENTIFIED PRECORDIAL BODIES

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Until the time of birth, and even for variable periods thereafter, small groups of fat cells, collectively known as primitive fat organs, may be found distributed more or less widely in the body. They may be so minute as to escape detection by the unaided eye, or they may be apparent as rounded or oval, stellate or otherwise angulated small bodies whose color, depending on the amount of pigment in them or on the degree of vascularity, varies from yellowish brown to bright cherry red. They occur especially in the fascial planes of the neck and the interscapular region, in the loose connective tissues of the precordium, and around the kidneys and adrenal capsules, but may be seen in practically any locality in the body where fat normally occurs. Those in the neck have been interpreted as a variety of gland belonging to the endocrine system.

In the body of an infant that I investigated post mortem primitive fat organs occurred in enormous numbers and were so distributed as to suggest a disseminated new growth. The subject was a boy aged 3 months, dead of inanition. In the loose connective tissue around the larynx, the esophagus and the thyroid gland, in the fascial planes between the muscles of the anterior and lateral portions of the neck, in the connective tissue between the pectoralis major and minor muscles and the coracobrachialis muscle on the left side, in the loose tissues of the precordium, in the parietal pleura corresponding to the lower edges of the fourth and fifth ribs on the right side, in the loose tissues immediately behind the sternum from end to end, and behind and in front of both kidneys, especially the left, was a quantity of cherry red tissue so distributed, practically wherever found, as to form a rough lattice work made up of oval or rounded glistening bodies from 2 to 5 mm. in diameter, arranged discretely or joined by strands of the same general appearance. This trellis-like distribution was particularly noticeable in the retrosternal region, in the precordium and around the thymus gland, where it was abundant. In the neck and between the superficial muscles of the thorax the tissue was found in branching chains. In the connective tissue of the right pleura the tissue formed chains which lay parallel with the edges of the ribs. The tissue in the peritoneum in front of the kidneys and in the capsules of the kidneys themselves was arranged in a coarse network. Behind the kidneys the tissue was clumped to form thick cordlike masses lying in the fascia of the psoas muscles. In specimens fixed in formaldehyde solution the reddish bodies described could be seen breaking up into small angulated masses loosely held together by connective tissue. Histologic examination showed the presence of innumerable rounded, oval or angulated, richly cellular small islands lying in a reticulum of loose connective tissue. The islands were composed of a complex network of injected capillaries, between which were groups of large polyhedral cells with moderately chromatic, centrally placed small rounded nuclei and finely granular pinkish cytoplasm. Sudan III showed the presence of numerous minute orange-red granules lying in the cytoplasm of many of the cells, while others were free from stainable fat.

In 1915 Pende [1] described what he believed to be a new gland of internal secretion (glandula insularis cervicalis). He found it in children and in puppies, in the form of from fifteen to twenty solid islands of cells, which he assumed to be of epithelial nature and which lay in the connective tissue around the thyroid and thymus glands. The cellular islands appeared as richly vascularized reddish bodies made up of sharply defined small cells of rounded or polygonal outline with a centrally placed vesicular nucleus, and a distinct cell membrane. The cytoplasm contained numerous small granules so closely packed as to give the cytoplasm a homogeneous appearance, the granules staining pinkish with eosin and responding to special stains for fat. Pende assumed that the granular and fatty substances in the cell body were the visible expres-

From the Laboratories of Pathology, Goldwater Memorial Hospital.

1. Pende, N.: Arch. f. mikr. Anat. **86**:193, 1915.

sion of an internal secretion. It requires but a glance at the several illustrations which accompany Pende's paper to convince one that the body described by him as a new member of the endocrine series is morphologically identical with the primitive fat organ. By this I do not mean to deny that the structure described by him is a

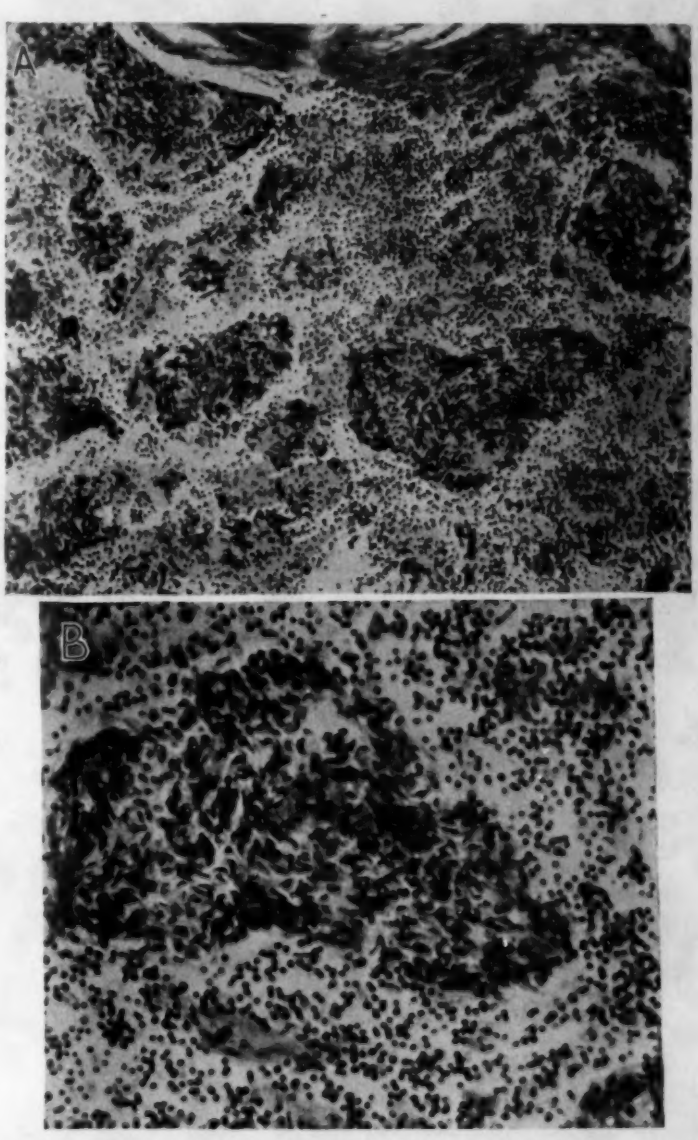

Fig. 1.—*A*, low power photomicrograph of unidentified precordial spindle cell bodies lying in a stroma sprinkled with lymphocytes. At the top is part of a thick bundle of collagenous fibers by which the precordial bodies were supported. Hematoxylin and eosin; paraffin; × 151.

B, high power photomicrograph showing one of the unidentified precordial spindle cell bodies surrounded by lymphocytes. At the lower left corner is a group of lightly staining capillary blood vessels in which the red cells are fused. Hematoxylin and eosin; paraffin; × 302.

gland of internal secretion. In fact, others have since offered the same suggestion as applied to all primitive fat organs.

The so-called interscapular or hibernating gland, first described by Merkle, is a paired fat organ, lobulated and irregular in shape but definite in outline, extending over a considerable area in the region of the shoulders and the sides of the neck. In the adult it is pinkish, but in the newborn it contains variable quantities of pigment and is brownish. The fat tissue of the hibernating gland is abundantly vascularized by capillary blood vessels. According to Bonnot,[2] it differentiates first into lobules composed of fixed polygonal cells, some of which become

2. Bonnot, E.: J. Anat. & Physiol. **43**:43, 1908-1909.

laden with minute fat particles. It then differentiates into lymphoid tissue which further differentiates into hemolymph nodes and lymph nodes. The interscapular gland is constant in mammals, and in man it is probably the homologue of the hibernating gland of rodents.

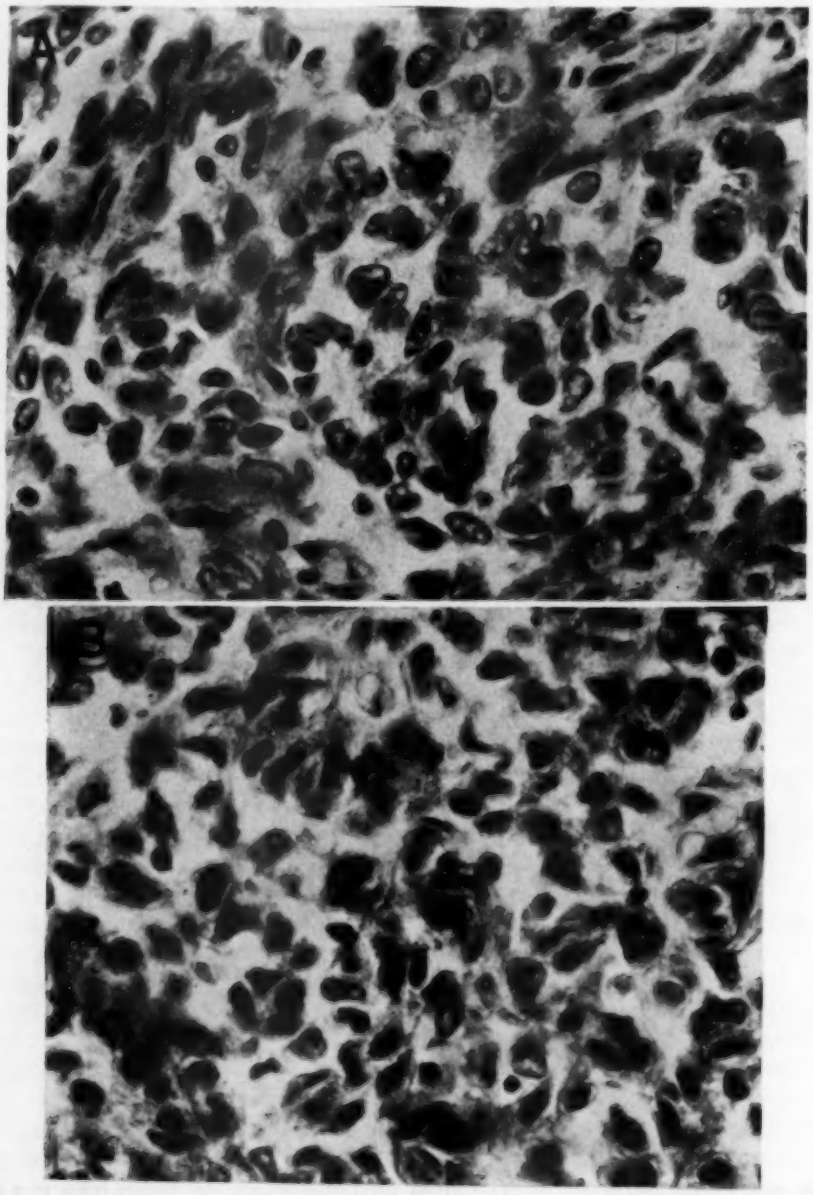

Fig. 2.—*A*, high power photomicrograph of tumor derived from unidentified precordial bodies, showing an admixture of long spindle cells and cells with rounded or ovoid nuclei, arranged irregularly. Hematoxylin and eosin; paraffin; ×776.

B, high power photomicrograph of another part of the tumor shown in *A* in which there is almost complete absence of long spindle cells. Hematoxylin and eosin; paraffin; ×776.

In certain lower animals that part of the gland which is provided with polygonal fat-laden cells has been shown to serve as a source of food supply during the period of hibernation. For example, Shattock,[3] referring to the results of his investigation of the fat in the hibernating gland of the hedgehog, stated that "during hibernation these reserves are used up and dwindle away, a residuum of connective tissue alone remaining, to be again replaced before the arrival of the next hibernating season." Others have voiced opinions to the effect that the fat in these localities

3. Shattock, S. G.: Proc. Roy. Soc. Med. **2**:208, 1908-1909.

seems not to be disturbed during hibernation or starvation. Bonnot, who shared the belief that the hemolymph nodes participate in the destruction of red cells, has shown that a part of the pigment in the fat of the hibernating gland is hemosiderin. The function of the lymphoid tissue in the hibernating gland appears to be the same as that of lymphoid tissue elsewhere. However true all this may be, that opinion is apparently sound which holds that the hibernating or interscapular gland is an organ primarily set apart for the storage of fat.

Inglis [4] described a solid tumor derived from the polygonal fat cells of the interscapular gland. The growth lay in the interscapular region to the left of the midline and on removal measured 11 by 8 by 5.5 cm. In the subcutaneous fat of the immediate vicinity were several small secondary nodules. The growth was the color of putty and was divided into lobules by connective tissue septums. Microscopically, it was composed of rounded or polygonal cells with centrally placed, moderately richly chromatic nuclei and foamy cytoplasm in which sudan III revealed innumerable minute fat particles.

As far as I am aware Inglis' tumor is the only solid growth thus far recorded which had its origin in a primitive fat organ. He also described a congenital cystic growth of the neck in a girl 6 weeks of age, arising presumably from lymph vessels in the interscapular gland. It consisted of three types of cysts, one containing clear fluid, one containing blood and one containing semifluid material.

4. Inglis, K.: J. Anat. **61**:452, 1927.

REPORT OF A CASE

I have recently encountered a sarcoma arising possibly from primitive fat organs. The growth was detected in the histologic examination of two nodules removed at necropsy from the precordial area of an emaciated man 72 years of age. The nodules were ovoid and measured 4 by 3 by 3 cm. They showed no connection with any of the other mediastinal structures and were easily removed. Their outer surfaces were covered with fat. On section they presented thick fibrous walls and were filled with whitish, apparently partly necrotic material.

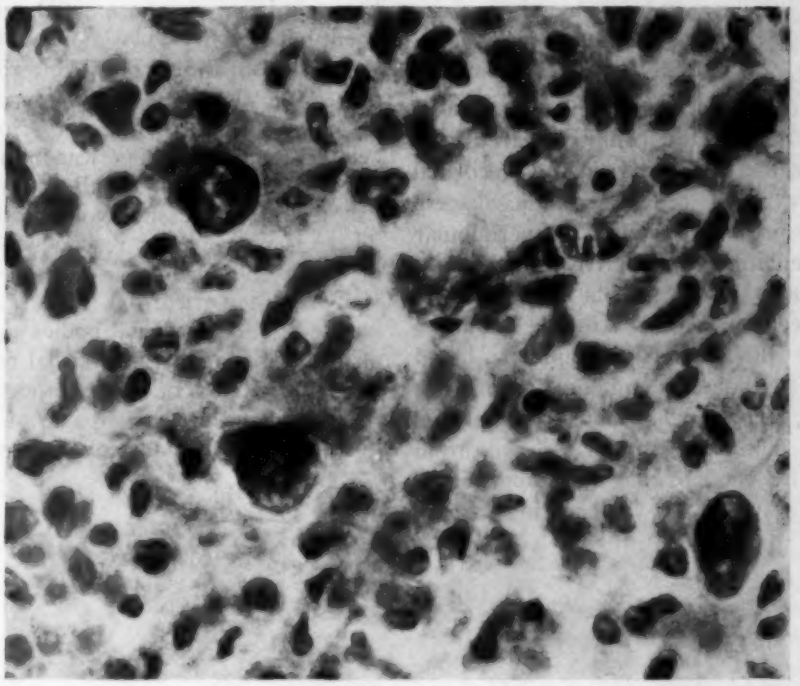

Fig. 3.—High power photomicrograph of same tumor as in *A* and *B* of figure 2, showing giant cells. Hematoxylin and eosin; paraffin; × 776.

Microscopically, they lay among thick bands of collagenous fibers. Resting on the surface of the collagenous bands and in the fibrofatty tissues in the immediate vicinity of the tumor were great numbers of rounded, oval or indented or otherwise irregularly outlined large and small islands or lobules of cells lying in a stroma which was richly sprinkled with lymphocytes and supplied with moderate numbers of capillary blood vessels. A few of the cells in the lobules were polygonal, but most of them were long, slender and spindle shaped, and their contours were well defined. The cytoplasm of both the spindle-shaped and the polygonal cells was finely granular and faintly acidophilic, and the nuclei were fairly dense and deeply staining. In frozen sections stained with sudan IV, minute granules of fat were detected in some of the polygonal cells. The spindle cells showed no stainable fat. In sections impregnated with silver delicate agryophilic supporting fibrils were seen.

The cells of the tumor presented no uniformity of size, shape or arrangement, although at times there was a vague suggestion of columnar or papillary or perithelial formations. Some of the tumor cells were long and spindle shaped, others were oval or rounded, and all of them were indistinctly outlined. The cytoplasm was abundant, finely granular and faintly acidophilic; the nuclei were rather deeply staining, and their chromatin content varied. Although polygonal cells were few, their cell membranes stood out sharply, and they were easily identified in frozen sections stained with sudan IV, in which the cytoplasm of some of them was found to be crowded with closely packed orange-red granules. Other polygonal cells did not react at all with sudan IV, nor was fat demonstrable in any of the spindle cells. Giant cells with richly chromatic rounded or twisted nuclei occurred in scattered areas. The tumor cells in many places invaded the interstices of the supporting collagenous bundles, where they could be seen as clumps or linear formations.

CONCLUSIONS

Histologically, the precordial bodies differ from the primitive fat organs in at least two particulars. First, in the primitive fat organs the prevailing cell is polygonal; in the precordial bodies the prevailing cell is spindle shaped. Second, in the precordial bodies the perilobular tissues are vascularized by relatively small numbers of capillary blood vessels lying among loosely scattered lymphocytes; in the primitive fat organs the distribution of capillary blood vessels is in the lobules and not around or between them, the capillary blood supply is infinitely more abundant than in the precordial bodies, and, with the possible exception of the so-called interscapular or hibernating gland, collections of lymphocytes are absent. That they may resemble one another is shown by the presence of stainable fat in some of the polygonal cells common to both.

If it is true that the endocardium is homologous with the intima of the aorta, the myocardium with the media and the pericardium with the adventitia, it is conceivable that the precordial bodies with their islands of spindle cells and diffuse accumulations of lymphocytes are pericardial rests homologous with the adventitia of the aorta, where small numbers of fibroblasts and minute lymphocytic foci are normally present. According to this view, it would seem to follow that in the case under discussion both the spindle cells and the lymphocytes must have undergone hyperplasia in response to the same causative agent but that hyperplasia of the spindle cells had progressed to the stage of cancerous transformation, while the lymphocytes remained passive as far as neoplasia is concerned. Spindle and giant cell sarcoma of the pericardium seems not to have been described. On the other hand, Zacher [5] has recorded a case involving the peritoneum. There is no apparent reason why the same sort of tumor should not arise in the pericardium as well as in pericardial rests. Whatever the nature of the precordial bodies may be, whether primitive fat organs, pericardial rests or independent formations of some other sort, the fact remains that they are capable of transformation into spindle and giant cell sarcoma.

5. Zacher, P.: Centralbl. f. allg. Path. u. path. Anat. **34**:313, 1923-1924.

INCIDENCE OF PRIMARY CARCINOMA OF THE LUNG WITH SPECIAL REFERENCE TO ITS INCREASE

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INTRODUCTION

Carcinoma of the lung [1] has become recognized as one of the common tumors in man—a distinction which man shares with mice and no other known species. In necropsy statistics from general hospitals on the frequency of occurrence of carcinoma it usually ranks third,[2] being exceeded by carcinoma of the stomach and carcinoma of the large bowel. It is rarely found in second place except when there is failure to consider the rectum as part of the colon, carcinoma of the large bowel being separated into two groups,[3] or when there are special selective factors,[4] or when there is total failure to mention the colon.[5] On the other hand, it is occasionally found in fourth place.[6] Among all malignant neoplasms, in contrast to those diagnosed as carcinoma, this is probably its correct position.

The recognition that primary carcinoma of the lung is among the common tumors is recent. Adler [7] in 1912 collected only 372 cases from the literature. In the second and third decades of the present century, and with increasing frequency thereafter, papers appeared describing series of 100 or more cases so that by 1933 Hruby and Sweany [8] were able to find 2,359 cases recorded in the literature and thought that this represented about half of those reported. Since then many additional ones have been placed on record, including those listed in table 1. That the reported cases represent only a small proportion of the whole is indicated by the estimation of Overholt and Rumel [9] based on data obtained from Louis Dublin that 15,000 persons die each year in the United States from carcinoma of the lung. Dorn [10] has recently arrived at a different, but high, figure. He estimated that slightly more than 8,000 new cases are diagnosed each year and that about 13,000 patients are under treatment at all times.

Incidence.—The reported incidence of carcinoma of the lung shows wide variation, depending on the source of the data. The mortality rate for the year 1935 in the United States is given by Gover [11] as 4.52 per hundred thousand of population. Brunn [12] collected 718 cases in 608,250 necropsies from 1872 to 1924, an incidence of 0.11 per cent. Rosahn [13] in 1930 found the incidence in 249,851 necropsies to be 0.42 per cent while the incidence among all cancers diagnosed as carcinoma was 4.6 per cent. Hruby and Sweany [8] in 1933 found carcinoma of the lung in 1,355 of 185,434 collected necropsies (0.73 per cent) in which the total number of tumors diagnosed as carcinoma was 22,712 (5.96 per cent). Simons [14] in 1937 summarized the data from the literature in detail and showed the great variation in various cities and countries.

A compilation of cases reports of which have been published in the recent literature from the United States is given in table 1. Many of these reports were made after the monograph by

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1. Throughout this paper the term "carcinoma of the lung" is used in preference to "bronchogenic carcinoma" because it is more inclusive and possibly more correct since it has not been proved that all tumors diagnosed as carcinoma primary in the lung arise from the bronchi.

2. (*a*) Jaffé, R. H.: J. Lab. & Clin. Med. **20**:1227, 1935. (*b*) Klotz, M. O.: Am. J. M. Sc. **196**:436, 1938. (*c*) Rosahn, P. D.: Arch. Path. **29**:649, 1940. (*d*) Turner, H. M., and Grace, H. G.: J. Hyg. **38**:90, 1938.

3. Rosedale, R. S., and McKay, D. R.: Am. J. Cancer **26**:493, 1936.

4. Brines, O. A., and Kenning, J. C.: Am. J. Clin. Path. **7**:120, 1937.

5. (*a*) Halpert, B.: J. A. M. A. **117**:1090, 1941. (*b*) Koletsky, S.: Arch. Int. Med. **62**:636, 1938.

6. (*a*) Wegelin, C.: Schweiz. med. Wchnschr. **72**: 1053, 1942. (*b*) Bonser, G. M.: J. Hyg. **34**:218, 1934. (*c*) Bauer, J. T.: Bull. Ayer. Clin. Lab., Pennsylvania Hosp. **3**:139, 1938.

7. Adler, I.: Primary Malignant Growths of the Lungs and Bronchi, New York, Longmans, Green & Co., 1912.

8. Hruby, A. J., and Sweany, H. C.: Arch. Int. Med. **52**:497, 1933.

9. Overholt, R. H., and Rumel, W. R.: J. A. M. A. **114**:735, 1940.

10. Dorn, H. F.: Pub. Health Rep. **58**:1265, 1943.

11. Gover, M.: Cancer Mortality in the United States: I. Trend of Recorded Cancer Mortality in the Death Registration States of 1900 from 1900 to 1935, Public Health Bulletin 248, Federal Security Agency, United States Public Health Service, 1939.

12. Brunn, H.: Arch. Surg. **12**:406, 1926.

13. Rosahn, P. D.: Am. J. M. Sc. **179**:803, 1930.

14. Simons, E. J.: Primary Carcinoma of the Lung, Chicago, Year Book Publishers, Inc., 1937.

Simons appeared. The table shows that carcinoma of the lung was found in 925 of 93,560 necropsies (0.99 per cent). The tumors with this diagnosis constituted 7.4 per cent of all tumors encountered in these necropsies, and 9.4 per cent of all those diagnosed as carcinoma. The incidence of carcinoma of the lung in all necropsies varies from 0.4 to 2.16 per cent and that among all tumors diagnosed as carcinoma, from 3.9 to 13.71 per cent.

as reported by Hsieh, Wang and Chang.[19] In Canada the range of variation is about the same as in the United States, according to Boyd,[20] Klotz[2b] and Menne and Anderson.[21]

Question of Increase.—Of special interest is the question whether carcinoma of the lung has increased. Most authors agree that it is seen more frequently now than formerly. If Brunn's[12] collected cases, from 1872 to 1924, are divided into three groups, the percentages

TABLE 1.—*Incidence of Primary Carcinoma of the Lung at Necropsies Reported in Recent Papers in the United States*

				Total Tumors		Tumors Diagnosed as Carcinoma		Tumors Diagnosed as Carcinoma of Lung			
Author	Institution	Years	Total Necropsies	No.	% of All Necropsies	No.	% of All Necropsies	No.	% of All Necropsies	% of All Tumors	% of All Tumors Diagnosed as Carcinomas
Bauer [2c]	Pennsylvania Hosp., Philadelphia	1899-1938	6,000	529	8.8	...	.....	32	0.53	6.05	....
Brines and Kenning [4]	Receiving Hosp., Detroit	1937	3,000	...	.....	...	.....	25	0.83	....	....
Frissell and Knox [37]	St. Luke's Hosp., New York	1900-1935	3,659	...	.....	588	16.1	39	1.06	....	6.63
Halpert [5a]	Charity Hosp., New Orleans	1931-1940	12,972	...	.....	...	.....	135	1.04	....	....
Jaffé [2a]	Cook County Hosp., Chicago	1928-1934	6,800	...	.....	871	12.81	100	1.47	....	11.47
Koletsky [5b]	Cleveland City Hosp., Cleveland	1927-1937	7,685	1,064	13.8	...	.....	100	1.30	9.31	....
MacCallum, W. G.: Tr. A. Am. Physicians **45**: 77, 1930	Johns Hopkins Hosp., Baltimore	1890-1930	11,254	...	.....	780	6.8	30	0.3	....	3.9
Matz [30]	Veterans Admin., Washington	1927-1937	7,398	...	.....	1,167	15.77	160	2.16	....	13.71
Menne and Anderson [21]	Univ. or Oregon, Portland	1928-1940	7,971	...	.....	...	.....	83	1.04	....	....
Olson [2e]	Boston City Hosp., Boston	1900-1934	7,893	...	.....	762	9.7	69	0.87	....	9.06
Perrone, J. A., and Levinson, J. P.: Ann. Int. Med. **17**: 12, 1942	Mercy, Hosp., Pittsburgh	1911-1939	2,604	...	.....	359	13.32	38	1.41	....	10.59
Rosahn [2c]	Yale University, New Haven, Conn.	1917-1937	4,114*	...	.....	435	10.6	43	....	....	9.9
Rosedale and McKay [3]	Buffalo City Hosp., Buffalo	1925-1934	4,670	466	9.9	...	.....	33	0.7	7.1	....
Wallace, W. S., and Jackson, H. G.: Texas State J. Med. **38**: 605, 1943	Univ. of Texas, Galveston	1942	5,000	546	10.9	...	.....	28	0.6	6.0	....
Weller [38]	Univ. of Michigan, Ann Arbor	1892-1927	2,450	...	.....	244	9.9	10	0.4	....	4.1
			93,560	2,605	11.1	5,206	11.3	925	0.99	7.4	9.4

* The subjects were all over 20 years of age.

Among recent reports from other countries are those of Fabris,[15] who saw pulmonary carcinoma in 150 of 10,000 necropsies in Venice in ten years, and von Glinski,[16] who reported 184 cases from Stettin in 1939, where carcinoma of the lung was seen in 0.89 per cent of all necropsies and 6.82 per cent of all cases of carcinoma. In Denmark Husted and Biilmann[17] found about the same percentage, while in Glasgow it was slightly higher according to Dick,[18] and in China it was only 0.8 per cent of all cases of carcinoma

for the incidence in all necropsies become 0.04, 0.24 and 0.21. Rosahn[2c] grouped necropsies into ten year periods and found a steady increase in carcinoma of the lung whether expressed in terms of all necropsies or of all cancers diagnosed as carcinoma. The same increase is seen in most of the data which Simons collected from the literature. Menne and Anderson[21] in 1941 gathered data on 33,945 necropsies performed in the Pacific Northwest from 1920 to 1940. Carcinoma of the lung was found 517 times (1.52 per cent of all necropsies). The increase in suc-

15. Fabris, A.: Acta, Union internat. contre cancer **3**:130, 1938.

16. von Glinski: Deutsches Arch. f. klin. Med. **185**: 73, 1939.

17. Husted, E., and Biilmann, G.: Acta path. et microbiol. Scandinav. **14**:141, 1937.

18. Dick, J. C.: Glasgow M. J. **134**:63, 1940.

19. Hsieh, C. K.; Wang, S. H., and Chang, F. C.: Chinese M. J. **58**:381, 1940.

20. Boyd, W.: Canad. M. A. J. **23**:210, 1930.

21. Menne, F. R., and Anderson, M. W.: J. A. M. A. **117**:2215, 1941.

cessive five year periods reported for two hospitals was slight but persistent. The death rate in the United States registration area from carcinoma of the lungs and pleura increased 3.7 times between 1914 and 1930, compared with an increase of 20 per cent for all forms of cancer combined.[11] Bonser [6b] collected data from forty-three general hospitals in thirty-five towns in fourteen countries. Six reported no increase (Innsbruck, Austria; Hamburg, Germany; Lund, Sweden; Leeds, England; Leningrad, Russia; Glasgow, Scotland), five reported a slight increase (Cologne, Germany; Magdeburg, Germany; Lemberg, Poland; Budapest, Hungary; Troppau, Czecho-Slovakia), while thirty-two reported a distinct increase. Bonser [22] in 1938 brought the reports from Great Britain up to date in a table which shows an increase for most of the hospitals.

Time of the Increase.—If there has been an increase in the incidence of carcinoma of the lung, when did it occur? On this point the reports in the literature show striking disagreement. According to the compilation of Brunn,[12] it occurred prior to 1916. In America Barron [23] found a big increase in the period from 1914 to 1921 over that from 1899 to 1918. Jaffé and Sternberg [24] reported the incidence of pulmonary carcinoma among all tumors diagnosed as carcinoma to be about as high (10.73 per cent) in Vienna in the period from 1915 to 1918 as Jaffé [2a] subsequently found it to be in Chicago in the years from 1928 to 1934 (11.47 per cent). At the Mayo Clinic Vinson [25] saw a marked increase during the decade 1925 to 1935. Menne and Anderson [21] mentioned an abrupt appearance of primary carcinoma of the lung (in 1926) in their autopsy experience in Portland. Olson [26] said that a big increase occurred at the Boston City Hospital in the years 1930 to 1934, Rice [27] reported an increase at the Wisconsin General Hospital beginning in 1930, and Halpert,[5a] at the Charity Hospital, New Orleans, showed an increase in the period from 1931 to 1940.

In other countries, Simpson [28] found a fourfold increase at the London Hospital between 1907 and 1925, with the greatest rise from 1918 to 1922. At St. Bartholomew's an increase began in 1920, according to Maxwell and Nicholson.[29] At Leeds, however, Bonser[6b] was unable to detect a significant increase prior to 1933, while at Edinburgh [30] the increase occurred between 1929 and 1934. Harbitz [31] said that an increase occurred in Norway after 1930. In Australia Harvey [32] saw an increase from 1930 to 1935, in China an increase began in 1936 [19] and in Denmark it was seen as early as 1924.[17].

The statement is commonly made that the increase occurred later in the United States than in Europe. This impression seems unwarranted when the vital statistics are examined.[11] Here an increase is shown beginning at least as early as 1914, and, according to Dorn,[10] it has continued to 1940, although at a diminishing rate.

Nature of the Increase.—It appears to be established that carcinoma of the lung has been seen with increasing frequency in recent years. The problem then becomes one of deciding whether this increase is apparent and relative or whether it is real and absolute. Opinions recorded in the literature are divided on this point. Rosahn [2c] concluded that the increase is real. With this opinion Harvey,[32] Maxwell and Nicholson,[29] Simpson, [28] White and co-workers [33] and others have been inclined to agree. On the other hand, Fried,[34] Jaffé,[2a] Harbitz,[31] Weller,[35] Hruby and Sweany [8] and others have stated that there has probably been no true increase. Others have held that their data are inconclusive. These include Kennaway and Kennaway,[36] Bonser,[22] El-Gazayerli,[30] Frissell and Knox [37] and Stein and Joslin.[38] Still a fourth group has expressed the opinion that the increase is both real and apparent. These writers include, among others, Matz,[39] Halpert,[5a] Husted and Biilmann [17] and Tripoli and Holland.[40]

Klotz [2b] has pointed out that Rosahn and Fried after using the same material came to opposite conclusions, as did Kikuth and Breckwoldt and also Passey and co-workers and Daguid. It is possible that this confusion arose

22. Bonser, G. M.: Acta, Union internat. contre cancer **3**:119, 1938.
23. Barron, M.: Arch. Surg. **4**:624, 1924.
24. Jaffé, R. H., and Sternberg, H.: Virchows Arch. f. path. Anat. **231**:346, 1921.
25. Vinson, P. P.: J. A. M. A. **107**:258, 1936.
26. Olson, K. B.: Am. J. Path. **11**:449, 1935.
27. Rice, C. M.: J. Lab. & Clin. Med. **21**:906, 1936.
28. Simpson, S. L.: Quart. J. Med. **22**:413, 1929.
29. Maxwell, J., and Nicholson, W. A.: Quart. J. Med. **24**:29, 1930.
30. El-Gazayerli, M.: J. Hyg. **36**:449, 1936.
31. Harbitz, F.: Norsk mag. f. lægevidensk. **98**:1451, 1937.
32. Harvey, C.: M. J. Australia **1**:565, 1936.
33. White, T. J.; Cohen, S.; Gnassi, A. M., and Price, P.: J. A. M. A. **118**:862, 1942.
34. Fried, B. M.: Medicine **10**:373, 1931.
35. Weller, C. V.: Arch. Path. **7**:478, 1929.
36. Kennaway, N. M., and Kennaway, E. L.: J. Hyg. **36**:236, 1936.
37. Frissell, L. F., and Knox, L. C.: Am. J. Cancer **30**:219, 1937.
38. Stein, J. J., and Joslin, H. L.: Surg., Gynec. & Obst. **66**:902, 1938.
39. Matz, P. B.: J. A. M. A. **111**:2086, 1938.
40. Tripoli, C. J., and Holland, L. F.: South. M. J. **33**:559, 1940.

because some of the data which are used were not adequate to prove the point either way. Macklin [41] after surveying the data and the methods used concluded that a real increase had not been proved.

That portion of the increase which is relative and apparent is explained by many factors including the following: (*a*) Better pathologic diagnosis, with a change in the pathologic criteria as to what should be included with primary carcinoma of the lung (small spindle cell and round cell tumors formerly called "oat cell sarcoma," many of the tumors called pleural endothelioma, and some tumors formerly thought to be metastases but now called primary carcinoma); (*b*) greater clinical interest and awareness, as well as improved diagnosis by roentgenograms, bronchoscopic biopsy and pulmonary puncture, and a greater tendency to follow the subsequent course of the disease and finally obtain permission for necropsy; (*c*) interest in surgical treatment, which causes the patients to congregate at centers where thoracic surgery is performed and where necropsies are subsequently made on many; (*d*) an increase, out of proportion to the aging of the general population, in the average age of the population from which the patients are drawn (Matz).

No adequate explanation has been offered for a real increase, if such occurs, for the causes of only a relatively small proportion of all cases of pulmonary carcinoma are known. The chemical action of inhaled radioactive dusts accounts for the induction of the Schneeberg tumors [42] but does not explain an increase in America. Kennaway and Kennaway [36] tried to find whether the increase was real and, if it was, to discover its cause by studying the death certificates of males from England and Wales. They investigated 8,808 cases of pulmonary carcinoma and found an increase from 361 deaths in 1921 to 1,553 in 1932. They concluded that while more cases were detected in recent years, the data were inadequate to show whether the increase was real. They found no increase in any occupational group sufficient to account for the rise. Dick [18] has suggested that as more cancers in superficial sites are cured, more cancers in deep organs have an opportunity to develop. Probably, however, most persons with pulmonary carcinoma have not previously had a superficial cancer.

It seems to be established that all cancer is increasing in accordance with the increase in life expectancy, but it is not fully established that carcinoma of the lung is increasing more than other forms.

Importance of the Increase.—The point is not merely of academic interest. If a real increase is occurring in this country now during a time when the racial composition is changing little, it means that a consideration of hereditary etiologic factors in this particular kind of tumor can be minimized and efforts concentrated on a search for etiologic factors in the environment. If such could be demonstrated and if they could be abolished, the problem of the control of pulmonary carcinoma would be partly solved by prophylaxis.

General Suitability of the Present Data.—There would be no point in entering this controversy with an additional paper unless the data presented were new and well founded. Factors which have hitherto often been omitted should be taken into account. It is believed that the records and the materials in the department of pathology of the University of Chicago, on which this paper is based, are exceptionally well suited for such a study for the following reasons: 1. They cover a long span, namely, from 1902 to the present, a period of over forty years. 2. They have a great subjective uniformity because from the beginning until comparatively recently the final opinion of every tumor was passed by one man, the late Dr. H. Gideon Wells. 3. The records, the microscopic sections and in some cases the gross specimens are available for restudy if any datum is questionable. 4. The records begin long before the date of the alleged increase in America, affording a long control period. 5. The data are such that all of the important factors known at the present time except one can be corrected for.

NATURE OF THE NECROPSY POPULATION [43]

This report is based on 5,515 consecutive necropsies performed by members of the department of pathology of the University of Chicago in the forty years, 1902 to 1941 inclusive. For some of the analyses 6,000 consecutive postmortem examinations made during the period from 1902 to July 1943 were used.

Sources.—From the beginning a few necropsies were performed each year at scattered small hospitals and for private physicians. Until 1927, however, the majority were done at the Cook County Hospital. In 1928 this service was discontinued, but a weekly necropsy service was begun and has continued to date at the Cook County Infirmary at Oak Forest, Ill. Both of these institutions provide care for indigent ill people of Cook County, including Chicago. The infirmary has a slower rate of patient turnover and a population of slightly older

41. Macklin, M. T.: Ann. Int. Med. **17**:308, 1942.

42. Pirchan, A., and Sikl, H.: Am. J. Cancer **16**:681, 1932.

43. Because future statistical studies on tumors are planned which will deal with this same series of necropsies, the composition and sources of the material are given in detail.

average age, which, however, is kept down by a tuberculosis unit. There is much overlap in patient populations because many are transferred from the hospital to the infirmary, increasing the number with chronic diseases at the latter. The percentage of deaths followed by necropsy has been low at both institutions during the years included here. The exact figures for the hospital, as well as for the other institutions to be mentioned, are published annually.[44] The selection of cases for postmortem examination was determined by legal availability of the body, due to failure of relatives to claim the body, rather than by special interest in a diagnostic problem. During the years under survey there was no special interest in or surgical attention to pulmonary carcinoma at either institution which would tend to concentrate cases there.

In 1916 a necropsy service was begun at the Illinois Central Hospital at Chicago; it has continued to date. This is a general hospital of 250 bed capacity which provides medical care for employees of the railroad of the same name as well as for the private patients of a large attending staff. The patients are about equally divided between these two sources. Autopsies have been made on about 30 per cent of the patients who have died.[44] The records disclose no recognizable preponderance of any disease. The staff is well balanced, and it is believed that despite the low percentage of necropsies no peculiar selective factor was exerted. While interest in the clinical diagnosis of pulmonary diseases has been active, there has been no surgical work on the lungs which would attract patients referred because of carcinoma and pad the figures.

In 1927 the University of Chicago Clinics were opened, adding a new source of necropsies, which since 1927 has contributed about 65 per cent of the annual total. These clinics include four hospitals with a total bed capacity of 525, of which 222 are general, 73 pediatric, 68 orthopedic and 162 obstetric. The patients are divided between free, part pay and full pay groups, the latter two groups predominating. They come from Chicago, its suburbs and the surrounding cities, country and adjacent states. The members of the professional staff are all specialized. This has selectively attracted certain diseases, unbalancing the necropsy records, as will be subsequently shown. Because the clinics always have a waiting list of patients who wish to enter the hospital no effort is made to keep patients as inpatients after treatment except those undergoing special observation. Postmortem examinations have consistently been made on about 80 per cent of all who die.[44] As far as is known there is no artefact introduced here due to selection of cases for necropsy. A vigorous attempt is made to obtain permission for postmortem examination after every death, and an annual survey of the failures discloses that they are distributed nearly equally in all services.

Variables.—Three changes were noted in the necropsy records of this department after the University of Chicago Clinics were opened in 1927: (*a*) There was a sudden and sustained increase in the annual number of necropsies from a previous maximum of 91 to an average of about 300 each year; (*b*) the sex ratio was changed by an increase in the percentage of females; (*c*) the percentage of necropsies in which tumors were encountered was increased. These changes must be taken into consideration when the data are used for statistical purposes.

Race.—Of the 5,515 subjects of autopsies, 5,176 (93.9 per cent) were white persons and 339 (6.1 per cent) were Negroes. The Negro population of Chicago is generally stated to be 10 per cent of the total. The figure given here (6.1 per cent), however, probably quite accurately represents the proportion of Negroes in the population from which the subjects of necropsies were obtained, because many of the white patients came from the suburbs and from the surrounding country, in both of which Negroes are uncommon.

Included with white persons are 6 Chinese, 17 Mexicans, 2 Filipinos, 2 East Indians, 2 Japanese and 1 American Indian. These numbers are too few for separate analysis or to have any appreciable influence on the total necropsy population. It is of interest that the 6 Chinese had among them three cancers diagnosed as carcinoma (one involving the nasopharynx and two the colon).

The remainder of the white population is too heterogeneous for subdivision. It includes descendants of immigrants and direct immigrants from all of the European national and racial stocks. It probably accurately represents the racial composition of this community. British

TABLE 2.—*Incidence of Primary Carcinoma of the Lung at Necropsies in Five Year Periods from 1902 to 1941, Inclusive, at the University of Chicago*

	All Necropsies			All Tumors				Tumors Diagnosed as Carcinoma of Lung			
Five Year Period	Number	Average Age	% in Females	Average Age	Number	% in Females	% of All Necropsies	Number	% of All Necropsies	% of All Tumors	Number in Males
1902-1906	187	40.9	21.9	56.0	22	31.9	11.6	2	1.1	9.1	1
1907-1911	171	41.3	18.7	52.4	23	30.4	13.4	1	0.6	4.4	0
1912-1916	334	45.6	14.1	51.9	50	18.0	15.0	1	0.3	2.0	1
1917-1921	319	44.5	18.8	51.8	73	13.9	22.9	3	0.9	4.1	3
1922-1926	414	44.4	25.1	51.0	75	12.0	18.1	5	1.2	6.7	4
1927-1931	1,087	46.2	34.3	53.8	350	37.4	32.2	29	2.5	8.3	21
1932-1936	1,467	44.9	34.9	51.7	447	34.8	30.5	33	2.2	7.2	29
1937-1941	1,536	45.6	36.1	52.1	621	38.5	40.4	53	3.4	8.6	41
Totals	5,515	....	31.3	....	1,661	34.1	30.1	126	2.3	7.6	100

44. Necropsies in the Hospitals of Chicago: Twenty-Fourth Annual Report, Proc. Inst. Med. Chicago **14**: 525, 1943.

and Germans predominated in the early immigration; later large numbers of Scandinavians, Italians, Slavs and Jews, as well as others, were added.

Sex.—Of the 5,515 subjects of autopsies, 3,791 (68.7 per cent) were males and 1,724 (31.3 per cent) were females. The ratio of the two sexes has not been constant. The proportion of males was even greater prior to 1927, at which time the opening of the University of Chicago Clinics tended to equalize the sexes. The changes in the sex ratio are given in table 2 and in chart 1. They are of importance in studies on types of tumor that are unequally divided between the sexes, such as carcinoma of the lung.

Age.—The average age of subjects of necropsies was 45.9 years in 1902 and 46.3 years in 1941. The record for five year periods through the intervening years is shown in table 2. This remarkable observation that there was only a slight increase in average life span of persons subjected to necropsy during a period of forty years is not explained by a relative increase in the number of infants and children. The observation still holds true if all necropsies on children under 1 year or under 12 years are omitted. While diphtheria, typhoid fever and tuberculosis have decreased remarkably or disappeared in recent years, bacterial endocarditis, glomerulonephritis and other diseases are seen oftener. This apparently accounts for part of the failure of increase in average age in this necropsy population.

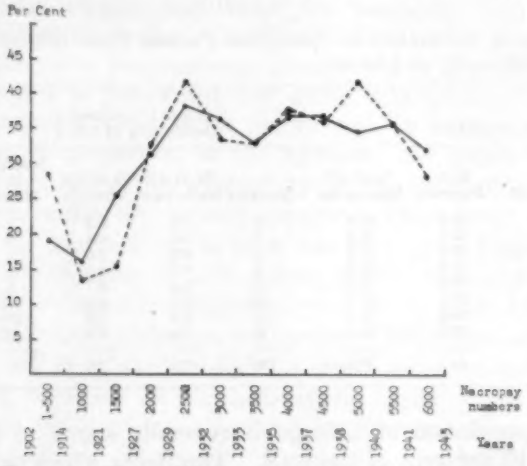

Chart 1.—The solid line represents the female subjects of necropsies; the broken line, the tumors in female subjects of necropsies.

The very slight increase in average age is of importance in studies on tumor incidence. It simplifies the analysis by virtually eliminating this factor as a variable.

Of the 5,515 subjects of necropsies, 332 were under 1 year of age and 607 were under 12 years. With the exception of a short time around 1927, when there was a decrease, the percentage of necropsies in children has been fairly constant.

Percentage of Necropsies at Which Tumors Were Disclosed.—The percentage of necropsies at which malignant tumors were observed increased. Until 1927 the increase was slow but it rose rapidly in that year from a previous level of about 20 per cent to 33 per cent. It reached the highest point in 1940, when 41.8 per cent of autopsies revealed cancer (table 2). Of the 5,515 subjects of necropsies, 30.1 per cent have had a neoplasm. This high incidence of tumors is contributed largely by the University of Chicago Clinics, where a high incidence of neoplasms also prevails in other departments. For example, about 25 per cent of surgical operations are performed for tumors. These facts obviously indicate a concentration of patients with tumors in the University clinics' population. For this study they mean that the incidence of pulmonary carcinoma will have to be stated in terms of the percentage of all tumors rather than of all necropsies.

Sex Ratio for Tumors.—The sex ratio for all tumors roughly has paralleled the sex ratio for all necropsies. Of all patients with tumors 34.1 per cent were females, while 31.3 per cent of all subjects of necropsy were females.

Because the number of tumors found each year in females was small during the early years, leading to wide fluctuations, graphs were made by collecting all necropsies into consecutive series of 500 each and determining the percentage of tumors in females in each series as well as the percentage of female subjects in all autopsies in each series. Six thousand necropsies performed from 1902 to 1943 were so treated, and the results are shown in chart 1. Here the close parallelism between the two curves is apparent.

Total Hospital Admissions and Total Deaths.—Data are not available on these two points, and the incidence of tumors, therefore, cannot be expressed in terms of percentage of these two factors.

METHODS OF ANALYSIS

A number of different methods were used for analyzing the data. By one the tumors were divided into consecutive series of 200 each, according to the method of Rosahn.[2e] By another all necropsies were divided into consecutive series of 500 each and the tumors compared in such series. By a third method the tumors in consecutive five year periods were compared. Each method had advantages as well as disadvantages. The results were essentially the same. The third method was finally adopted for most of this paper because the results can

be more easily compared with those of other authors, who have nearly all used this method. The other methods, however, will be used to emphasize certain points.

Definition of Term "Malignant Tumors."—As used in this report the term "maligant tumors" includes the following: tumors diagnosed as carcinoma (1,224); tumors diagnosed as sarcoma (90); fatal intracranial tumors (181); tumors occurring in lymphatic diseases, including leukemia, aleukemic leukemia, lymphosarcoma, Hodgkin's lymphogranuloma and related diseases (140), and miscellaneous tumors, including those diagnosed as malignant melanoma, malignant mixed tumor, mesothelioma and malignant carcinoid (27)—a total of 1,661.

The figures given in the foregoing paragraph include a few cured and subclinical malignant neoplasms. To be included these tumors must have been beyond question correctly diagnosed. The small numbers so included do not materially influence the results.

The problem arises as to whether all malignant neoplasms should be used as the basis of comparison or only those diagnosed as carcinoma. Opinion in the literature is about equally divided on this point (table 1). In this paper all tumors are used because it was believed desirable to use intracranial tumors as one control group to make a special point.

If all malignant tumors rather than only all tumors diagnosed as carcinoma are used for the analyses, it becomes important to retain the necropsies on persons under 20 years of age because of the bone tumors, the tumors diagnosed as adrenal neuroblastoma, those diagnosed as renal adenomyosarcoma, tumors of the brain and others in this age group. Unless the number of necropsies on infants under 1 year old is relatively large or shows wide fluctuations from time to time, it need not be excluded.

INCIDENCE OF CARCINOMA OF THE LUNG

One hundred and twenty six of the tumors encountered in the 5,515 necropsies were diagnosed as carcinoma of the lung, an incidence of 2.3 per cent. By referring to table 1 it can be seen that this incidence is comparatively high. The incidence was 2.6 per cent in persons over 1 year of age and 2.7 per cent in those over 12 years.

These tumors diagnosed as carcinoma of the lung constitute 10.3 per cent of all the tumors classed as carcinoma, of which 1,224 were found in the 5,515 necropsies. They are 7.6 per cent of all malignant neoplasms (total, 1,661). Pulmonary carcinoma stood third in frequency among all types of carcinoma and fifth among all types of tumors, being exceeded by carcinoma of the stomach, intracranial tumors, carcinoma of the colon and lymphatic tumors, in that order.

Of these 126 pulmonary cancers, 100 occurred in males and 26 in females, a proportion of about 3.8 to 1. If a correction is made for the unequal sex ratio in the necropsy population (69.7 per cent males to 31.3 per cent females), the ratio becomes 1.8 in males to 1 in females.

Six of the pulmonary cancers were found in Negroes and 120 in white persons. After correction the figures show the disease to be 1.3 times commoner in white persons than in Negroes, an observation which is probably not significant in view of the small number of Negroes on whom necropsies were made. The figures show, however, that carcinoma of the lung does occur commonly in Negroes, a point previously made by Quinland,[45] who reported 3 of the 6 cases mentioned here. He found nothing in them different from carcinoma of the lung in white persons.

The average age of these 126 persons was 54.9 years. The youngest was 32 and the oldest 86. Others in whom the disease occurred at an early age were 32, 32, 33, 33, 36, 37, 38 (3 persons) and 39 years old.

QUESTION OF INCREASE IN CARCINOMA OF THE LUNG

The number of cases of pulmonary carcinoma found in consecutive five year periods shows a striking increase (table 2). When expressed as a percentage of all necropsies the increase between 1902 and 1941 is about threefold.

Because the number of tumors diagnosed as pulmonary carcinoma, as well as that of other tumors, was small in the early years, leading to wide fluctuations in percentage, the data were reanalyzed by another method. The 6,000 necropsies from 1902 to 1943 were divided into twelve consecutive series of 500 necropsies each, and the numbers of tumors diagnosed as pulmonary carcinoma in the series were used to construct chart 2 *A*. It shows a definite increase throughout the necropsy series as well as through the years.

IS THE INCREASE IN CARCINOMA OF THE LUNG RELATIVE OR REAL?

While an increase in the number of cases of carcinoma of the lung in these necropsies is shown in the preceding section, it remains to be determined whether this increase is apparent or real. Since the percentage of necropsies which showed tumor increased during the years under study, the incidence of carcinoma of the lung must be expressed in percentage of all tumors

45. Quinland, W. S.: South. M. J. **35**:729, 1942.

rather than in percentage of all necropsies, as was done in the preceding section.

This has been done in table 2, in which the percentage of all tumors diagnosed as carcinoma of the lung shows first a fall followed by a rise to its former level.

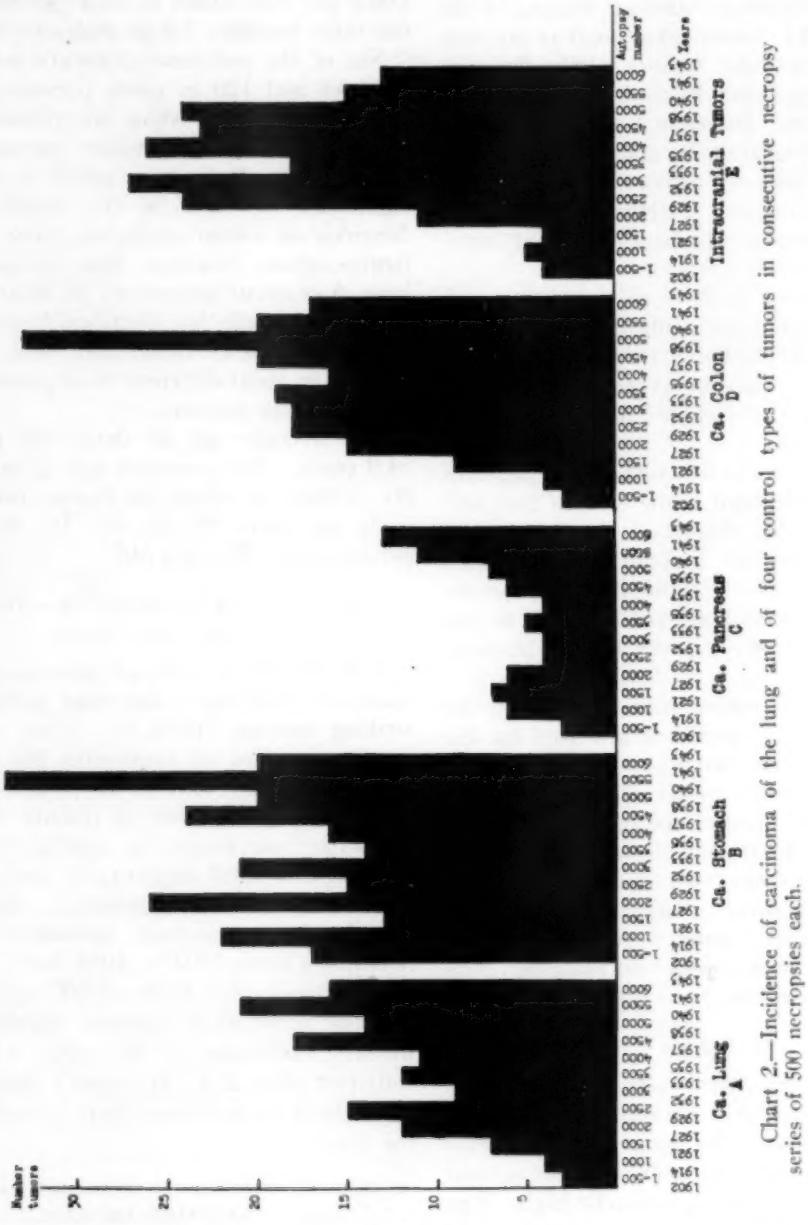

Chart 2.—Incidence of carcinoma of the lung and of four control types of tumors in consecutive necropsy series of 500 necropsies each.

Control types of tumors were next studied. There were four common types, all having practically a 100 per cent mortality in the past, occurring in both sexes, having a low incidence of error in diagnosis on postmortem examination, the cases of which all came to necropsy at the same hospitals and were studied by the same staff: carcinoma of the stomach, carcinoma of the colon, carcinoma of the pancreas and intracranial tumors. For none of them is an increase generally claimed. Three of them were encountered in more numerous instances than carcinoma of the lung and the fourth (carcinoma of the pancreas) was half as common. Two of them had about the same sex ratio as pulmonary carcinoma but for the other two the percentage in females was higher. These data are shown in tables 3 and 4. Chart 2 shows that the increases in carcinoma of the colon and intracranial tumors were even more precipitous than that in pulmonary carcinoma, while gastric and pancreatic carcinoma each increased only slightly. Table 3 shows that, expressed in percentage of all tumors, carcinoma of the pancreas and carcinoma of the stomach decreased, and carcinoma of the colon and intracranial tumors increased. Expressed in terms of

percentage of all necropsies carcinoma of the colon and intracranial tumors increased, while carcinoma of the stomach and carcinoma of the pancreas remained about constant.

Again, because the small number of cases in the early years may lead to wide fluctuations, the data were recalculated by another method. Curves of incidence for the control types as well as for pulmonary carcinoma in consecutive series of 500 necropsies each were calculated in percentage of all tumors, and the results obtained were used to construct chart 3 *A*. The results are in close harmony with those in tables 2 and 3. Carcinoma of the lung showed no preliminary fall but only a gradual rise; gastric cancer decreased at first and then remained stationary; carcinoma of the colon and intracranial tumors increased even more than did pulmonary carcinoma, and carcinoma of the pancreas showed a drop followed by a rise.

Before this increase in pulmonary carcinoma, as well as that in two of the control groups, can be accepted as real two additional factors must be taken into consideration. These are (*a*) the average age of all persons at necropsy and (*b*) the change in sex ratio in the necropsies.

As previously mentioned, the average age of this necropsy population has increased very little since 1902 and not at all since 1911. The data are presented in table 2. The stabilized average age level antedates (1912-1916) the alleged increase in pulmonary carcinoma, which is usually considered to have taken place between 1920 and 1935, and it antedates the increases in the five types of tumor now under analysis (table 3 and chart 3 *A*). The increase in these types is therefore not explained on the basis of an aging necropsy population.

A correction must be made for the change in sex ratio in the necropsy population, which occurred principally after 1927, because the sex ratio is different for these types of tumors. The data are shown in table 4.

The ratio of males to females was approximately the same for carcinoma of the lung, the stomach and the pancreas and therefore the influence of this factor on the incidence of the three types of carcinoma should have been the same. These types were about twice as common in males as in females. When the sex ratio changed (about 1927) a divergence between the curves for these three types of tumor should be evident in comparison with the curves for carcinoma of the colon and intracranial tumors, both of which were more common in females than in males. As the percentage of females in the necropsy population increased, the latter two types should have increased at a relatively faster rate than carcinoma of the lung, the stomach and the pancreas. That is exactly what the data show.

The situation is perhaps made clearer by analyzing the tumors in each sex separately. For this purpose all tumors were divided into two groups according to sex of patients, and the incidence of carcinoma of the lung, as well as that of each of the control types, was calculated in terms of percentage of all tumors in that sex in each series of 500 necropsies. The curve constructed from the results for males is shown in chart 3 *B* and that for females in chart 3 *C*.

The curves show that the increase in pulmonary carcinoma was present only in males and that it occurred abruptly about 1927, a time when the nature of the necropsy population underwent great change.

The increase in intracranial tumors was found in both sexes, the increase in cancer of the colon was mainly in males, and the drop in gastric carcinoma was almost entirely in males.

TABLE 3.—*Incidence of Control Types at Necropsy*

Five Year Period	Carcinoma of Stomach				Carcinoma of Colon				Intracranial Tumors				Carcinoma of Pancreas			
	Number	% of All Necropsies	% of All Tumors	Number in Males	Number	% of All Necropsies	% of All Tumors	Number in Males	Number	% of All Necropsies	% of All Tumors	Number in Males	Number	% of All Necropsies	% of All Tumors	Number in Males
1902-1906	6	3.2	27.3	5	1	0.5	4.6	0	0	0.0	0.0	0	0	0.0	0.0	0
1907-1911	6	3.5	26.1	6	1	0.6	4.4	0	3	1.7	13.0	3	1	1.7	4.4	1
1912-1916	7	2.1	14.0	6	2	0.6	4.0	2	2	0.6	4.0	1	6	1.8	12.0	6
1917-1921	20	6.3	27.4	18	3	0.9	4.1	2	4	1.3	5.5	4	2	0.7	2.7	2
1922-1926	11	2.7	14.7	10	7	1.7	9.2	5	4	1.0	5.3	3	7	1.7	9.3	7
1927-1931	45	4.1	12.9	35	35	3.2	10.0	16	38	3.5	10.9	23	10	1.0	2.9	7
1932-1936	49	3.3	10.9	35	51	3.5	11.4	34	67	4.6	14.9	41	13	0.9	2.9	10
1937-1941	80	5.2	12.9	62	75	4.9	12.1	48	63	4.1	10.1	35	24	1.5	3.5	18
	224	4.1	13.5	177	175	3.2	10.5	107	181	3.3	10.9	110	63	1.1	3.8	51

TABLE 4.—*Total Numbers and Sex Ratios for Tumors Diagnosed as Pulmonary Carcinoma and Control Types*

	Total Number			Female : Male	
	Tumors	Female Patients	Male Patients	Ratio	Corrected Ratio
Carcinoma of lung.....	136	36	100	1:3.8	1:1.7
Carcinoma of stomach	224	47	177	1:3.8	1:1.7
Carcinoma of colon....	175	68	107	1:1.6	1:0.7
Intracranial tumors....	181	71	110	1:1.6	1:0.7
Carcinoma of pancreas	63	12	51	1:4.3	1:2.0

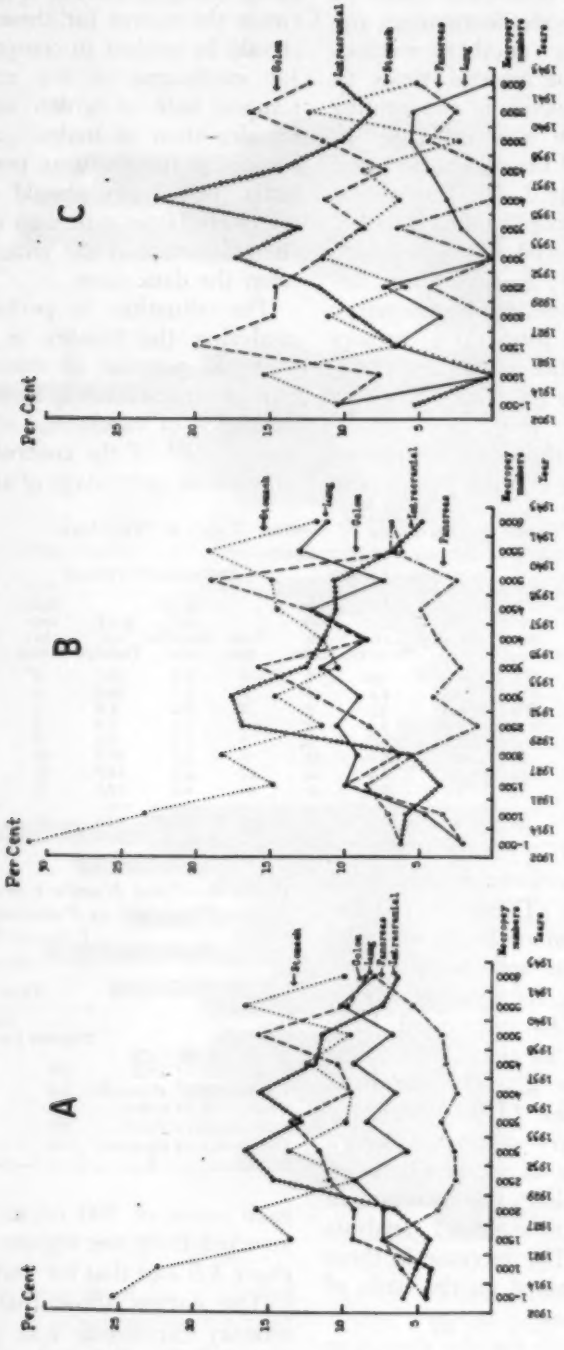

Chart 3.—*A*, incidence of carcinoma of the lung and of four control types of tumor in terms of percentage of all tumors. *B* and *C*, the same as found for the male and the female sex respectively.

These changes were confirmed in every respect when all tumors were divided into consecutive series containing 200 tumors each and then further divided into the two sex groups. This result was to be expected because the proportion of all tumors found in each sex has been practically constant.

COMMENT

The data presented show that carcinoma of the lung has been seen oftener in recent years than formerly, a feature shared with all of the types of tumor used as controls. This increase persists when the incidence of pulmonary carcinoma is expressed in percentage of all necropsies, but it almost disappears when it is expressed in percentage of all tumors; the increase which remains is found only in males. Thus the increase seen in the crude figures is largely explained by an increase in tumors of all kinds in the necropsy population and partly by a shift in the sex ratio in this same population. The increase, therefore, is largely apparent and not real.

One factor remains uncorrected. By its very nature it cannot be corrected. This is a shift in the type of patients admitted to the hospitals. In the hospital from which about two thirds of these cases were drawn the clinical attending staff is highly specialized. It is probable that some patients, such as victims of pulmonary carcinoma, were referred to them as their special skills and interest became known. This would result in a relatively greater increase in these diseases in the hospital population, and also in the necropsy population if these diseases carried a high mortality.

Intracranial tumors were selected as one of the control groups because they illustrate this point so well. The upswing in the incidence of intracranial tumors in these necropsies began when a large neurosurgical service was opened in the University of Chicago Clinics, and the decrease began when the size of this service was reduced. The total number of intracranial tumors in this necropsy population is probably much greater than in that of the average general hospital—another manifestation of the same special interest.

It is quite possible, and even probable, that a similar factor exists with respect to carcinoma of the lung at these clinics and that it accounts for the slight increase in pulmonary carcinoma which persists after the other corrections are made. Prior to 1936 there was great interest at the University of Chicago Clinics in massive doses of roentgen radiation for carcinoma of the lung,[46] and since then the increasing interest in pulmonary surgery has probably attracted patients with carcinoma of the lung; so that probably the increase in this disease is all apparent and not real in these data.

The increase in intracranial tumors is unquestionably explained on the basis of special interest of the clinical staff. The increase in carcinoma of the colon, which was also greater than that in carcinoma of the lung and nearly as great as that in intracranial tumors, is probably explained on the same basis. The department of general surgery has always had great interest in that form of cancer.

Although the rate for all neoplasms in percentage of necropsies is exceptionally high, that for carcinoma of the lung in percentage of all tumors, while among the highest so far reported, is not excessive. The only other recent data from Chicago are those of Jaffé,[28] who found the rate for carcinoma of the lung to be 11.47 per cent of all tumors diagnosed as carcinoma (compared with 10.3 per cent reported here) at the Cook County Hospital. Halpert[47] reported on the tumors from this department for one decade but made no corrections and began the study after the time of the increase (1927).

SUMMARY

In the forty years from 1902 to 1941, inclusive, primary carcinoma of the lung was noted in 126 of 5,515 necropsies performed by members of the department of pathology of the University of Chicago. The incidence was 2.3 per cent of all necropsies, 7.6 per cent of all malignant tumors and 10.3 per cent of all tumors diagnosed as carcinoma. It was fifth in frequency among all types of tumor and third among types of carcinoma.

The incidence of carcinoma of the lung expressed in terms of percentage of all tumors showed a slight increase in males but not in females. The increase was less than that shown for two types of tumors used as controls, namely, carcinoma of the colon and intracranial tumors, but greater than that for two other control types, carcinoma of the pancreas and carcinoma of the stomach.

Corrections were made for changes in the annual number of necropsies, in the sex ratio of all necropsies, in the percentage of neoplasms in all necropsies and in the average age of the necropsy population. The only known variable not subjected to correction (of necessity), a change in the nature of patients admitted to the hospitals, could easily account for the slight increase in carcinoma of the lung. It is believed that in this material there has been a slight apparent but no real increase in primary carcinoma of the lung.

46. Steiner, P. E.: Arch. Int. Med. **66**:140, 1940.

47. Halpert, B.: Cancer Research **1**:900, 1941.

INFECTIOUS MONONUCLEOSIS

REPORT OF A FATAL CASE WITH AUTOPSY

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BETHLEHEM, PA.

Owing to the extremely low mortality from infectious mononucleosis, there have been no previous opportunities to study this disease adequately from the standpoint of pathology.

In 1885 Filatov, in Russia, described an idiopathic type of lymphadenopathy of children. In 1889 Pfeiffer [1] called the disease glandular fever and established it as a distinct clinical entity.

Numerous epidemics of the disease have been reported. The first to be recorded in this country was that studied by West [2] in 1896. He reported 96 cases in children. The disease was considered one of childhood, but in 1908 Terflinger [3] reported 150 cases in adults, demonstrating for the first time that the disease may affect adults of any age.

In 1920 Sprunt and Evans [4] described "infectious mononucleosis," believing it to be a new disease entity. In 1921 Tidy and Morley [5] demonstrated that glandular fever and infectious mononucleosis were identical.

The diagnosis of the disease was placed on a firm foundation in 1932 by Paul and Bunnell,[6] who discovered the presence of agglutinins and hemolysins for sheep erythrocytes in the serum of patients having infectious mononucleosis.

There have been many excellent clinical and hematologic studies of this disease, and descriptions can be found in the standard texts of hematology and medicine. The mononuclear cells found in the blood and the tissues are peculiar and probably characteristic. Osgood [7] called attention to the more or less diagnostic fenestrations in these cells.

From the clinical standpoint the most impressive features of infectious mononucleosis are its protean manifestations, its extremely variable severity and its almost invariably favorable termination. The common symptoms and signs may include from one to several of the following: enlargement of lymph glands, fever, malaise, sore throat, headache, backache, anorexia, abdominal pain, splenic enlargement, hepatic enlargement, gastrointestinal upsets, chills, maculopapular cutaneous lesions, membranous pharyngitis, cerebral complications, hematuria and jaundice. Other symptoms have been reported. The serum of patients with this disease may give false reactions in serologic tests for syphilis. To the clinical picture one must add the characteristic changes in the blood cells and the positive heterophil antibody test. Without the latter the diagnosis may be questionable in some cases.[8]

The disease is most common in children and young adults. It has not occurred in Negroes so far as known. There is no seasonal or occupational incidence. The disease has been reported from North America, Europe, the Orient, Australia, British Guiana and Manila, P. I. It occurs in both epidemic and sporadic form.[8]

The following case is that of a patient with infectious mononucleosis who died in the fourth week of the disease from rupture of the spleen. Similar cases of rupture of the spleen due to this disease were reported by King and by Friesleben.[9] In these cases the spleens were removed surgically and the patients recovered.

REPORT OF CASE

M. R., a patient of Dr. E. W. Gilchrist, of Bethlehem, Pa., was a 22 year old white woman, a housewife, formerly a nurse and a native of Vermont. She was seen by her physician for the first time on July 18, 1942. She had a fever, her temperature ranging from 99 to 103 F. There were large discrete lymph nodes in the posterior cervical and the inguinal regions.

From the Pathological Laboratory, St. Luke's Hospital.

1. Pfeiffer, E.: Jahrb. f. Kinderh. **29**:257, 1889.

2. West, J. P.: Arch. Pediat. **13**:889, 1896.

3. Terflinger, F. W.: J. A. M. A. **50**:765, 1908.

4. Sprunt, T. P., and Evans, F. A.: Bull. Johns Hopkins Hosp. **31**:410, 1920.

5. Tidy, H. L., and Morley, E. B.: Brit. M. J. **1**:452, 1921. Tidy, H. L.: Lancet **2**:180 and 236, 1934.

6. Paul, J. R., and Bunnell, W. W.: Am. J. M. Sc. **183**:90, 1932.

7. Osgood, E. E.: Proc. Soc. Exper. Biol. & Med. **33**:218, 1935. Osgood, E. E., and Ashworth, C. M.: Atlas of Hematology, San Francisco, J. W. Stacey, Inc., 1937.

8. Kracke, R. R., and Garver, H. E.: Diseases of the Blood and Atlas of Hematology, Philadelphia, J. B. Lippincott Company, 1937. Houser, K. M.: Pennsylvania M. J. **46**:1173, 1943. Templeton, H. J., and Southerland, R. T.: J. A. M. A. **113**:1215, 1939.

9. (*a*) King, R. B.: New England J. Med. **224**: 1058, 1941. (*b*) Friesleben, M.: Deutsche Ztschr. f. Chir. **173**:45, 1922; cited by Downey, H., and Stasney, J.: Folia haemat. **54**:417, 1936.

July 20 the blood count showed 17,700 leukocytes and 4,500,000 erythrocytes. The hemoglobin content was 13 Gm. There were 6 per cent nonfilamented polymorphonuclears, 8 per cent filamented polymorphonuclears and 88 per cent mononuclears. July 22 a second blood count gave 23,800 leukocytes—7 per cent polymorphonuclears and 93 per cent mononuclears. Before hospitalization this nurse took her own temperature and found that it would rise to 102 or 103 F. in the afternoons and would be down to a normal figure in the mornings. One day previous to hospitalization she experienced nausea and vomiting, but these symptoms disappeared and did not recur.

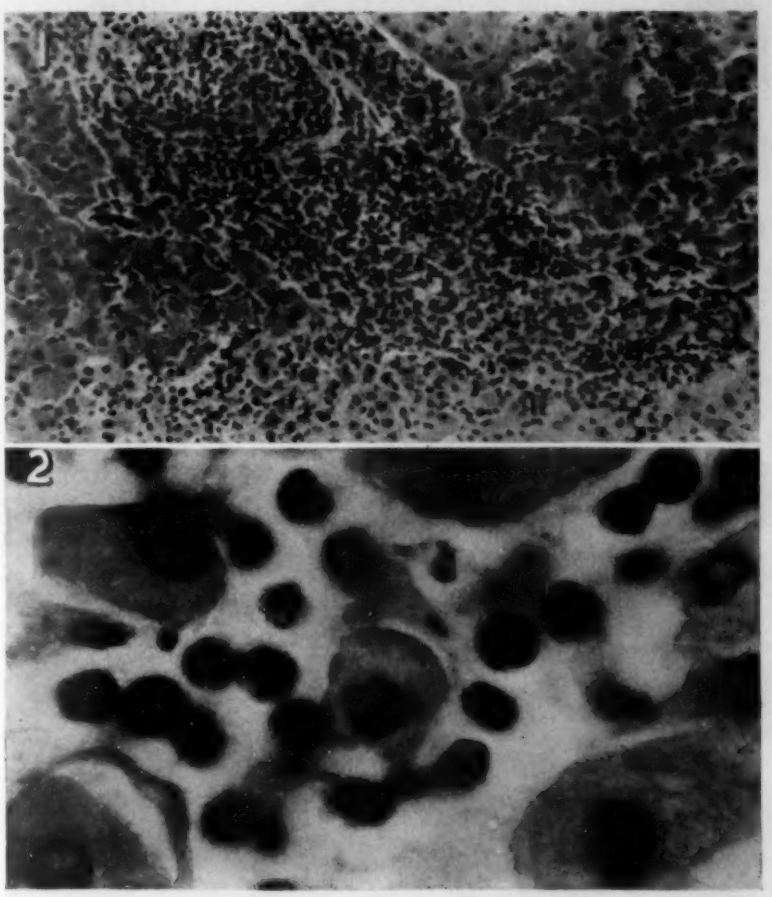


Fig. 1.—Liver; × 250; hematoxylin-eosin stain. Bile duct area of mononuclear infiltration and necrosis. Note dilated sinuses containing mononuclear cells. Two mitotic figures may be seen in the upper right portion of the field.

Fig. 2.—Liver; × 2,250; hematoxylin-eosin stain. Dilated and edematous sinuses containing mononuclear cells. Note indentation of some of the nuclei, also swollen hepatic and Kupffer cells. A liver cell at the lower right shows a mitotic figure.

The patient was admitted to the hospital July 24, 1942 with a diagnosis of infectious mononucleosis, complaining of sore throat, general malaise and fever of three weeks' duration. Previously she had been perfectly well. Her personal history was entirely irrelative except for the usual diseases of childhood. On admission she had a temperature of 100 F., a pulse rate of 93 and a respiratory rate of 22. The blood pressure was 105 systolic and 70 diastolic. The tonsils had been removed. The throat appeared moderately red and inflamed. The soft palate was covered with small mucoid blisters. The edge of the liver could be felt on inspiration and was tender. The spleen also was palpable for 2 to 3 fingerbreadths below the costal margin; it was slightly tender. Shotty discrete lymph glands were palpable in the neck, axillas and inguinal and popliteal regions.

In the hospital her temperature rose to 102 F. July 25. The next two days the highest points reached were 101.7 F. and 100 F., respectively. From July 24 to 28 the pulse rate ranged between 84 and 112. The respiratory rate was quite stable, varying from 20 to 24.

Because of the sore throat, the patient was given sulfathiazole July 26, but the following day this treatment was discontinued when it was noted that the patient had considerable anorexia.

July 28 the patient said that she felt weaker than usual, and her color was pale. This was at 10:30 a. m. At 11:40 a. m. she suddenly went into a condition of severe shock with a rapid, weak pulse and a pale, cold, damp skin. There was profuse diaphoresis. The blood pressure dropped to 50 systolic and 30 diastolic. An intravenous injection of isotonic solution of sodium chloride and dextrose was given. During the next thirty minutes the blood pressure rose to 90 systolic and 60 diastolic; then it began to fall rapidly again.

In the meantime the abdomen had become quite tense and distended.

At 12:25 p. m. the patient was given 500 cc. of plasma intravenously. Following this a second injection of plasma was ordered, but before it could be started, she grew weaker rapidly and died at 1:30 p. m. There had been a consultation, but the patient was not considered strong enough for any surgical procedure, and the possibility of a rupture of the spleen was hardly entertained.

or monocytes and cells of the lymphatic series. This is a matter of great convenience in differential counts in this disease, especially when one considers the frequent difficulty in trying to classify the large and peculiar mononuclear cells that are present.)

Dr. C. Merrill Leister, who saw this patient in consultation, furnished a blood smear from his files. He interprets the characteristic cells of this disease as being of lymphatic origin. He noted that they showed the characteristic fenestrations pointed out by Osgood,

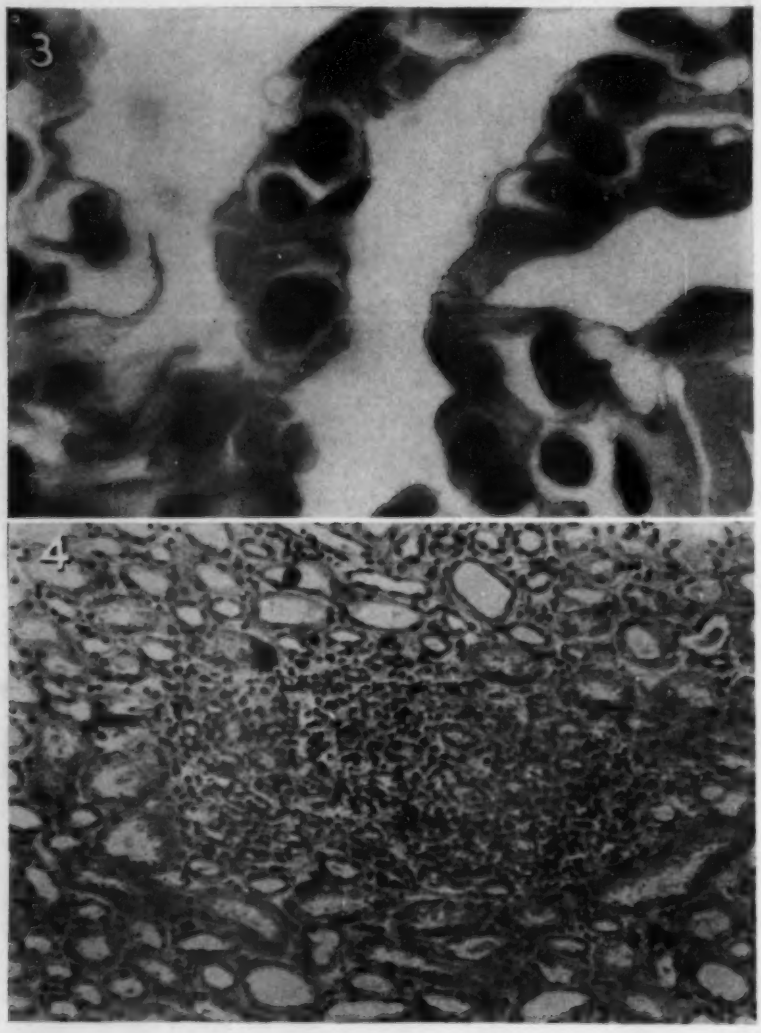


Fig. 3.—Lung; ×2,250; hematoxylin-eosin stain. Dilated and edematous capillaries containing mononuclears, some with indented nuclei. Occasional erythrocytes can be seen. The bronchial epithelium (not shown) is greatly swollen.

Fig. 4.—Kidneys; ×250; hematoxylin-eosin stain. Focal infiltration of leukocytes. Note shrunken tubules in focus, lined by flattened epithelial cells.

July 25 the heterophile antibody test showed agglutination of the sheep cells in a titer of 1:640. Urinalysis showed a trace of albumin but no other abnormality. A blood count showed 4,500,000 erythrocytes and 19,000 leukocytes; the hemoglobin content was 12 Gm. There were 7 per cent filamented polymorphonuclears, 4 per cent nonfilamented polymorphonuclears and 89 per cent mononuclears. It was noted that the platelets appeared normal in number, some being of large size. (The term "mononuclears" is used in this record to include both large mononuclears and his differential count of 300 cells is as follows: neutrophils 6, eosinophils 0, basophils 1, monocytes 2, lymphocytes 43, abnormal lymphocytes 48. These abnormal lymphocytes are larger than the normal lymphocytes; they often have fine azure granules, and their nuclei are sometimes indented. There are, however, hematologists who prefer to classify these peculiar cells as monocytes.

At the postmortem examination the abdomen was found to be filled with blood. The spleen was approximately three times the normal size. It was very

soft in consistency and brown, "like chocolate pudding." There was a rupture of the capsule about 7 or 8 cm. in length. Splenic substance could be scooped up in the hand "like so much mush." There were no other significant findings.

No bacteriologic studies were done during life or at autopsy, but there were no clinical or pathologic observations to indicate that common bacteria played any part in the case. However, this possibility cannot be conclusively excluded.

Histologic Observations.—(*a*) Liver: Scattered throughout were small to large-sized foci of the characteristic cells. The sinuses were dilated and contained morphonuclears and occasional eosinophils. There was a considerable number of cells of the lymphocytic series, but mature lymphocytes were scarce. The endothelial cells of sinuses and blood vessels were swollen. The sinuses were edematous, so that many of the Kupffer cells were separated from the adjacent liver cell cords. The small bile ducts were lined by swollen epithelial cells, so that their lumens were diminished. Some lumens appeared to be obliterated. These ducts showed pericellular edema in many cases, so that the duct epithelium was separated from its basement membrane. In general, the foci were well outlined. They contained occasional residual degenerated liver

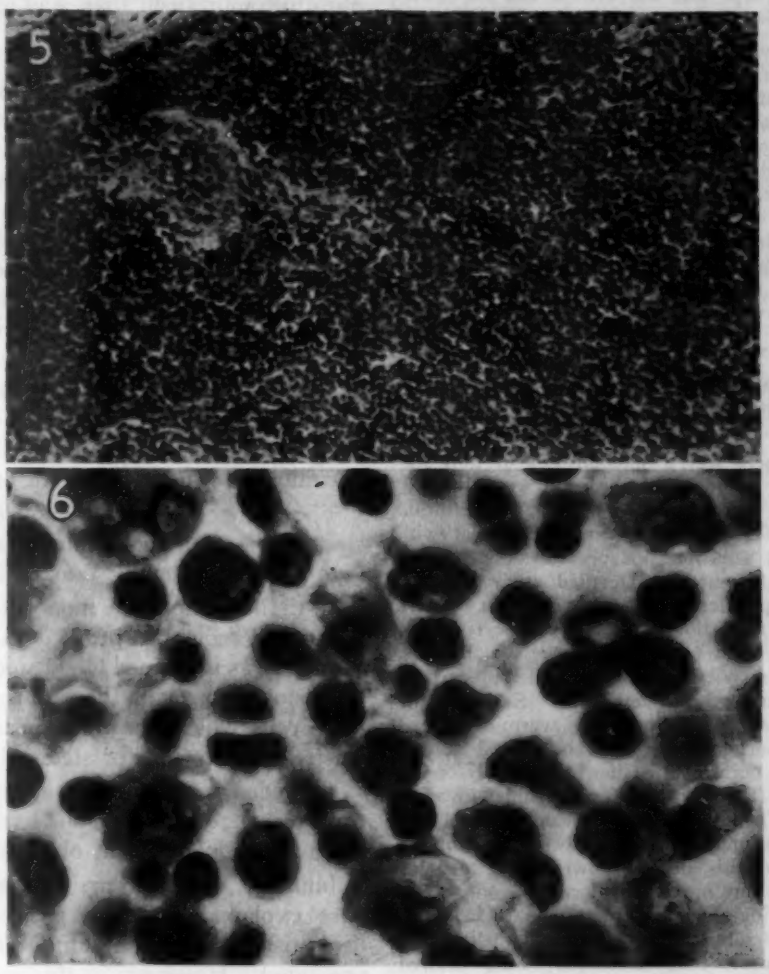


Fig. 5.—Spleen; × 250; hematoxylin-eosin stain. Note loss of follicular architecture and cellularity.

Fig. 6.—Spleen; × 2,250; hematoxylin-eosin stain. Detail of mononuclear cells showing variation in size. Reticuloendothelial and other cells are also present (see text). Some cells show "fenestrations."

numbers of the same types of cells seen in the foci, but hardly any red cells. The most extensive cellular infiltrations were perilobular, and they were especially prominent in the portal areas. However, there were smaller scattered foci of cell infiltration which showed no particular relationship to the lobular structure of the liver. The picture formed by the extensive infiltrations resembled that seen in leukemia. Examination of the foci with the oil immersion lens showed that they were made up of numbers of swollen reticuloendothelial cells (Kupffer cells) and numerous cells of the mononuclear type. There were occasional neutrophilic polycells. There were destruction and disappearance of most of the liver cells in the foci of infiltration. Around these foci one could see liver cells containing mitotic figures, and the liver cells were generally swollen and quite large. There was no vascular congestion or infiltration by red blood cells. Although the infiltrations as seen with low power resembled those of leukemia, it became apparent with the oil immersion lens that the detailed cellular picture is somewhat distinctive.

(*b*) Kidney: In the medullary portion there were scattered foci similar to those in the liver, but they were not as numerous. They were not found in the

cortical portions in this case. In these foci there was necrosis of the tubules. Shrunken remnants of tubules were seen. They were atrophic and were lined by flattened epithelium. In many of them the epithelium had disappeared entirely. The cells of the surrounding tubules showed mild to severe degrees of cloudy swelling, but they did not show regenerative activity as did the corresponding liver cells. The foci were rather sharply circumscribed. There was no diffuse infiltration between the foci as observed in the liver. The cells, however, were similar to those seen in the liver, but there were fewer of the reticuloendothelial type and there were more eosinophils. There was no interstitial hemorrhage and the vessels and capillaries contained only a few erythrocytes.

(*c*) Lungs: There were moderate emphysema and mild intra-alveolar edema. No intra-alveolar cellular exudate was seen. The alveolar capillaries were distended and in a few places showed congestion with erythrocytes. However, the great majority of them were distended and apparently in many cases obstructed by the large characteristic mononuclears. The capillaries appeared to contain more of these leukocytes than of erythrocytes. In addition there were some scattered perivascular and interstitial foci of mononuclear cells. Observation with the oil immersion lens showed that these leukocytes were of the same types as those found in the foci in the other organs. The alveoli were remarkably free of "heart failure cells." The bronchial walls were moderately infiltrated by mononuclear cells, and the lining epithelium was greatly swollen but not significantly exfoliated.

(*d*) Spleen: There was a subtotal loss of follicular architecture. The sinuses and the pulp were literally packed with the peculiar large lymphocytes and reticulum cells. The tissue was so cellular that with low power it had a neoplastic appearance not unlike that observed in lymphoma. Detailed study revealed slight fibroblastic proliferation of the reticulum. There was no syncytial formation. Small lymphocytes were few. There were great numbers of large lymphocytes, clasmatocytes and monocytes. Certain cells appeared to be lymphoblasts and others stem cells. Some of the cells showed mitotic figures. There were occasional neutrophilic polymorphonuclears and occasional plasma cells. There were occasional eosinophils, usually of the mononuclear type. There was moderate cellular invasion of the capsule. There were only occasional red blood cells. The reaction appeared to have been most intense in the spleen, and this organ showed more cellular pleomorphism than any other and more than one would expect from studying the blood picture in these cases. There were considerably more reticuloendothelial cells in the crowded sinuses than elsewhere. They showed some phagocytic activity. These cells, as well as some others, contained round, oval or fusiform "fenestrations." It is suggested that such structures may be stain-resisting bodies, possibly virus inclusions, and not fenestrations as they have been described in connection with blood studies. These stain-resisting bodies are translucent and not transparent as seen in these tissue cells. Another view held is that the "fenestrations" are artefacts and not necessarily diagnostic.

(*e*) Heart: The heart muscle appeared remarkably free of cellular infiltrations. It showed the acute changes common to many infectious diseases. It did not show vascular congestion.

(*f*) Appendix: The lymph follicles of the appendix did not show the cellular infiltrations observed in the spleen and elsewhere.

(*g*) Other tissues: Unfortunately, lymph nodes were not obtained at the autopsy or before death, and sections of other tissues were not available.

The histologic observations were in general agreement with those reported in the spleen, the marrow, the tonsils, the lymph nodes and the appendix by Warren (in King's [9a] paper), Gall and Stout,[10a] Pratt,[10b] Fox [10c] and Straus [10d] except that, as in the liver, the spleen and the lung, they were not entirely focal. Indeed, in the spleen they were quite diffuse and showed more pleomorphism than was expected.

COMMENT

It is altogether likely that foci of mononuclear infiltration as described in the foregoing report will be present to some extent in one or more types of tissue or organ in every case of infectious mononucleosis. The fact that these infiltrations are found in some tissues and not in others, together with their morphologic character, suggests strongly that they represent the reaction to foci of infection and that they are not merely a mechanical overflow of mononuclear leukocytes from the blood stream. The microscopic observations in this case suggest that the patient had hepatitis, nephritis, splenitis and pneumonitis of a characteristic and peculiar type.

The changes seen in the spleen of the patient seem to be of a more intense and diffuse type than those reported by Warren in King's paper.[9]

In tissue sections the monocytes cannot be distinguished from the peculiar large lymphocytes as readily as in stained blood smears. In addition the lesions, especially those in the spleen, contain large cells which one might classify as lymphoblasts, clasmatocytes or stem cells.

The edema, the cloudy swelling and the infiltrative changes found in the liver and the spleen explain the enlargement of these organs.

Jaundice, which occurs in some cases, is likewise explainable on the basis of diffuse focal hepatitis such as was found in the case now presented. However, in this case there was no jaundice. Whether or not jaundice develops depends no doubt on the extent and the intensity of the hepatic involvement. This seems to be a somewhat better hypothesis than that of the swelling of lymph nodes in the region of the bile ducts—at least in those cases in which enlargement of the liver is present. Then, too, it is reported that patients with jaundice do not have clay-colored stools. Jaundice has been reported

10. (*a*) Gall, E. A., and Stout, H. A.: Am. J. Path. **16**:433, 1940. (*b*) Pratt, C. L. G.: Lancet **2**:794, 1931. (*c*) Fox, H.: Am. J. M. Sc. **173**:486, 1927. (*d*) Straus, R.: Am. J. Clin. Path. **12**:295, 1942.

in cases of infectious mononucleosis by a number of observers.[11]

According to Thelander and Shaw,[12] "nephritis or bleeding from the kidney has been described in apparently authentic cases of the disease." The scattered foci of necrosis in the kidney with the characteristic mononuclear infiltration which I have described tend to confirm the clinical finding of nephritis in some cases.

Symptoms referable to the central nervous system have been reported by Thelander and Shaw,[12] by Landes, Reich and Perlow[13] and by Epstein and Dameshek.[14] I have no nerve tissue on which to report. One can readily imagine foci of mononuclear infiltration in the central nervous system or perhaps merely edematous and obstructed capillaries such as I demonstrated in the lung.

Bernreiter[11e] reported a case showing both jaundice and bronchial asthma. The changes which I have demonstrated in the lungs might possibly cause asthma-like symptoms in some cases. Bernreiter expressed the belief that these symptoms were caused by swelling of lymph glands around the bile ducts and the bronchial tree.

It is suggested that cases of mesenteric lymphadenitis and spontaneous rupture of the spleen, as reported by surgeons, could well be investigated to determine whether or not these conditions might be due to infectious mononucleosis. To these conditions one might add jaundice of undetermined origin and cryptogenic fevers generally. At least a heterophil antibody test and a cytologic study of the blood in these cases would do no harm.

SUMMARY

This study indicates, as generally held, that infectious mononucleosis is an acute or a subacute infectious disease of unknown cause having protean clinical and pathologic manifestations.

The focal character of the lesions as described in the liver and the kidney with mononuclear infiltration, reticulocyte proliferation and necrosis suggests an acute infectious granulomatous process. The disease appears to be a generalized infection with specific localization in one or more of the tissues or organs of the body. Certain lesions have formerly been described in the lymph nodes, the spleen, the tonsils and the marrow. To these have been added the changes in the liver, the kidneys, the spleen and the lungs of a patient proved to have the disease.

Infectious mononucleosis may occasionally run a fatal course. Previously fatal cases may not have been diagnosed. Three cases of rupture of the spleen resulting from this disease are now on record. One of the patients died; the other 2 were saved by surgical intervention.

It has been demonstrated that infectious mononucleosis may involve the parenchymatous organs as well as the lymphatic system.

11. (*a*) Carlile, T., and Blackford, J. M.: Northwest Med. **41**:137, 1942. (*b*) Chapman, A. A., and Chapman, J.: Southwestern Med. **24**:200, 1940. (*c*) Mason, V. R.: California & West. Med. **29**:187, 1928. (*d*) Mackey, R. D., and Wakefield, E. G.: Ann. Clin. Med. **4**:727, 1926. (*e*) Bernreiter, M.: J. Kansas M. Soc. **39**:513, 1938.

12. Thelander, H. E., and Shaw, E. B.: Am. J. Dis. Child. **61**:1131, 1941.

13. Landes, R.; Reich, J. P., and Perlow, S.: J. A. M. A. **116**:2482, 1941.

14. Epstein, S. H., and Dameshek, W.: New England J. Med. **205**:1238, 1931.

EFFECT OF LONG-CONTINUED ADMINISTRATION OF AN ESTROGEN ON THE SEX ORGANS OF MICE WHICH HAVE PASSED THE REPRODUCTIVE PERIOD

R. J. CROSSEN, M.D., AND LEO LOEB, M.D.
ST. LOUIS

As a general rule, the incidence of cancers, except those which originate during embryonal life, increases with increasing age. This may be attributed to the summation of cancerigenic stimuli, which have a chance to act on the various tissues more frequently and longer with increasing age of the subject. There is in addition the possibility that in older tissues there may be a factor which predisposes them to cancerous transformation; this factor in some instances may consist in the retardation of the processes of reparation after injuries at an older age, with the result that regenerative changes are longer continued. Certain cancers show their maximum incidence at a somewhat earlier age; this is true of some cancers of the secondary sex organs. The incidence of these cancers is presumably influenced by the activity curve of the ovarian hormones that specifically affect certain tissues of the secondary sex organs. These hormones cease to function before old age has been reached. Moreover, clinicians have observed hyperplasia of the endometrium in women during the menopause, and they have suggested that there may be a connection between this hyperplasia, due probably to the action of estrogen, and the development of carcinoma of the fundus of the uterus, which is liable to occur during this period of life or soon afterward. Of interest in this connection is the finding of Crossen and Hobbs [1] that 60 per cent of women with uterine carcinoma still continued to menstruate after the age of 50 years, while among a large number of women not affected by uterine carcinoma menstruation occurred in only 15 per cent at this late age. These observations support the conclusion that the long-continued action of ovarian hormones may be of significance in the origin of carcinoma not only in the mammary gland, the cervix and the vagina but also in the fundus of the uterus.

As to the age factor in the spontaneous mammary carcinoma of mice, as a general rule the tumors appear earlier the higher the incidence of this type of cancer in a certain strain. However, there were some families in which there was a dissociation between the incidence of carcinoma and the age at which carcinoma appeared. In the latter cases the genetic factors determining the incidence of mammary carcinoma were apparently distinct from the factors determining the age at which carcinoma developed. The existence of these two types of age factors was confirmed in experiments with hybrids between a strain with a high tumor rate and a strain with a low tumor rate. In general the incidence of carcinoma increased with increasing age up to a certain point; in the oldest age class a decrease in the incidence took place. If the effects of endogenous ovarian hormones were intensified by administering an estrogen to mice of strains varying in their tendency toward spontaneous development of cancer, the latent period which corresponded to the preparatory period was shortened.[2]

As can be seen from this brief review, the relation between age and cancer incidence is only partially understood. Under these conditions it seemed to be of special interest to study in animals which have passed the reproductive period the growth reactions of the sex organs to large amounts of an estrogen administered over longer periods and to analyze the various growth processes which might occur and the relation of these processes to the formation of cancerous tissue. The latter may be conceived of as the end stage of originally normal proliferative activities which

This investigation was aided by a grant from the International Cancer Research Foundation.

From the Laboratory of Research Pathology, Oscar Johnson Institute, Washington University School of Medicine.

The experimental part of this research was carried out by R. J. Crossen previous to June 30, 1941, when this laboratory was discontinued. The investigation, including the microscopic examination, was continued by the second author.

1. Crossen, R. J., and Hobbs, J. E.: J. Missouri M. A. **32**:361, 1935.

2. (*a*) Loeb, L.: J. Cancer Research **2**:135, 1917; (*b*) Am. J. M. Sc. **159**:781, 1920; (*c*) J. Cancer Research **6**:197, 1921; (*d*) Am. Naturalist **55**:510, 1921. (*e*) Suntzeff, V.; Moskop Kirtz, M.; Blumenthal, H. T., and Loeb, L.: Cancer Research **1**:446, 1941. (*f*) Tureen, L. L., and Loeb, L.: J. Cancer Research **13**:1, 1929.

take place under intensified stimulation of tissues, provided the latter possess genetically determined tendencies to respond to such stimulation with growth processes. There exists a relation between the effects of the stimulative and the genetic or constitutional factors which may be expressed approximately by the equation $H \times S = C$.[3] Proliferative processes in the mammary gland under the influence of hormones have been considered in previous papers,[4] and in other earlier papers references have been made also to precancerous and cancerous changes which take place in the vagina and cervix of mice.[5]

However, a certain difficulty has to be faced in experimenting with older mice. It is difficult to obtain a large number of such mice belonging to an inbred strain, and the death rate among them will in all probability be considerable if they are subjected to injections of an estrogen over longer periods. For these reasons the number of mice which we could use for the purpose of this investigation was smaller than we had expected. Nevertheless, certain observations regarding the growth processes in the secondary sex organs and the analysis and classification of the various proliferative changes with regard to their relation to precancerous and cancerous conditions are of considerable interest and may contribute to the understanding of the factors which are involved in the origin of tumors.

After exclusion of mice which died before the experiment was ended or which died during the night and were found in a condition which made them unsuitable for microscopic study, there were left 16 mice, 13 of which belonged to strain CBA, while 2 belonged to strain C57 and 1 to strain AKA. In these three strains the tendency toward formation of mammary carcinoma is low. The ages of these animals at the time of examination ranged between approximately 18 and 24 months except for the AKA mouse, which was only 16 months old at the end of the experiment. All of the animals had received weekly injections of 100 or 200 rat units of estradiol benzoate. Dr. Erwin Schwenk, of the Schering Corporation, supplied this estrogen. The period of the injections ranged between two and one-half and seven and three-quarters months. As in our previous investigations, the ovaries were studied in serial sections, while many sections were made through various parts of the vagina, the cervix, the uterus and the mammary glands of each mouse. Mice belonging to strains CBA and C57 which were used in earlier experiments and in addition 25 female mice belonging to strain CBA, ranging in age between 15 and 31 months, in which an almost complete examination of the sex organs was made, served as controls. Only some of the animals in the latter series were referred to in previous papers.

OBSERVATIONS

Ovaries.—There was marked reduction in number or entire loss of follicles; their place was taken by various types of interstitial gland cells, spindle cell connective tissue and ducts which developed either from the germ epithelium or from medullary ducts. A corpus luteum was observed in 1 of the 13 mice of strain CBA and in the AKA mouse. There was no notable difference in the ovaries of the experimental mice as compared with the controls. These findings suggest that the production of endogenous estrogen had largely ceased in these ovaries.

Mammary Glands.—Carcinoma had developed in the mammary glands of 3 of the 8 breeding mice and 1 of the 5 virgin mice of strain CBA. Precancerous changes were observed in 1 virgin mouse and in 2 breeding mice. In all of these animals, as well as in 1 of the C57 mice and in the AKA mouse, the mammary tissue was well developed; many groups or lobules of acini were present also in virgin mice, and in 4 virgin mice secretion was found. These conditions differed from those found in the controls: Among the 11 virgin controls in the main only ducts were noted, although in 3 a few isolated acini may have formed around the ducts. No tumors developed in this group. Among the 14 breeding controls 4 had mammary tumors and 1 a precancerous area, while in 6 breeding controls the mammary gland consisted merely of ducts, with the possible formation of a few isolated acini in 1 instance. In the estrogen-treated mice the mammary glands had therefore developed to a higher degree than in the nontreated controls; it may be concluded that the effects of the exogenous estrogen were superimposed on the action of endogenous hormones, which in the controls resulted in carcinoma only in the breeding mice. In the latter

3. Loeb, L.: (*a*) J. Cancer Research **8**:274, 1924. (*b*) Loeb.[2b] (*c*) Loeb.[2d]

4. (*a*) Loeb, L., and Suntzeff, V.: Arch. Path. **32**: 739, 1941. (*b*) Loeb, L.; Suntzeff, V.; Blumenthal, H. T., and Moskop Kirtz, M.: ibid. **33**:845, 1942. (*c*) Loeb, L.; Burns, E. L.; Suntzeff, V., and Moskop, M.: Am. J. Cancer **30**:47, 1937. Loeb.[2e] Loeb.[2d] Suntzeff and others.[2e] Tureen and Loeb.[2f]

5. (*a*) Allen, E., and Gardner, W. U.: Cancer Research **1**:359, 1941. (*b*) Gardner, W. U.; Allen, E.; Smith, G. W., and Strong, L. C.: J. A. M. A. **110**:1182, 1938. (*c*) Gardner, W. U., and Allen, E.: Yale J. Biol. & Med. **12**:213, 1939. (*d*) Loeb, L.; Burns, E. L.; Suntzeff, V., and Moskop, M.: Proc. Soc. Exper. Biol. & Med. **35**:320, 1936. (*e*) Overholser, M. D., and Allen, E.: ibid. **30**:1322, 1933. (*f*) Suntzeff, V.; Burns, E. L.; Moskop, M., and Loeb, L.: Am. J. Cancer **32**:256, 1938.

the tumor formation, which represents a change of old age, was due essentially to the long-continued action of the endogenous ovarian hormones, which presumably led in an intermediate stage to a state of sensitization of the mammary gland in which other, less specific stimuli also could exert growth-stimulating effects. It is of special interest that all of the treated mice in which tumors developed and 2 of the mice with precancerous changes had been treated weekly with 200 rat units of estrogen and that the effects of injections of 100 rat units were much less marked, a further indication that the exogenous estrogen had contributed to the cancerous or precancerous transformation.

Vagina.—The following effects were noted: 1. In all the animals the wall of the vagina was lined by squamous epithelium, often covered by keratin and sometimes also by keratohyalin. In no instance did the epithelium consist of only two layers, as did that over wide areas of the vagina in many animals of the control series; but adjoining the skin near the entrance of the vagina a transitional zone of squamous epithelium was found also in the controls. The responsiveness of the epithelium to growth stimuli decreased from the vagina to the cervix; the thickness of the squamous epithelium was diminished in the cervix. The difference in the character of the epithelial surface layer of the vagina and cervix between the experimental and the control mice must be attributed to the action of the exogenous estrogen.

2. In the experimental mice the squamous epithelium of the cervix penetrated into the beginning of the uterus; it undermined or pushed aside and destroyed the uterine cylindric epithelium of the surface and often made its way into the adjoining glands; however, the extent to which this penetration took place varied in different animals. Whereas in the control mice such invasion occurred in a few instances, it was present in all the experimental animals, this effect being due in the former to the endogenous estrogen and in the latter to the combination of the endogenous and the exogenous estrogen. This condition represents a competitive struggle between the squamous and the cylindric epithelium, which are both stimulated by the hormones, and in the struggle the former is shown stronger than the latter, at least for some time. Not only the proliferative processes in the epithelium but also the cell movements enter into this competition, both activities being stimulated by the hormones. Such an invasive competition is analogous to that between white and pigmented epidermis in the guinea pig after transplantation of a piece of pigmented skin into a defect of white skin.[6] This tendency of the stimulated vaginal cervical epithelium to extend over neighboring areas is probably related to the factors which cause the increased invaginations and foldings of the epithelium in the vagina and cervix when large amounts of estrogen are administered, or which takes place spontaneously with advancing age during the reproductive period[5f]; on the other hand, if atrophic processes occur in the vagina and cervix, such as are found so frequently in very old mice, this change may be reversed and there is a tendency toward partial obliteration of the invaginations and straightening out of the wall of the vagina.

3. Especially in somewhat older mice, the stroma of the vagina and cervix is invaded by solid processes of the surface epithelium, which may represent a further development of the invaginations already mentioned; usually these invasive processes remain moderate, but sometimes they progress to a precancerous state. If we exclude from consideration the zone of the transitional squamous epithelium which adjoins the skin, there were small or medium-sized solid processes reaching into the stroma in a number of the controls; they were found in various places in the vagina or the cervix. In only 1 or 2 of the mice were these processes somewhat more extensive, but even then they did not reach a typical precancerous condition. Much more marked were the corresponding changes in the mice to which estrogen had been administered. Eight of the 13 mice of strain CBA showed precancerous or nearly precancerous and usually multiple changes in the vagina or the cervix; they occurred either in several places in one of these organs or in both. In 4 of the animals the downgrowth of the epithelium was associated with loss of keratin and of the upper squamous epithelial layers under the influence of mechanical factors. As in our previous observations,[5f] this was found exactly at the site where the downgrowth took place; in neighboring regions, where the epithelium had its normal thickness, no downgrowth was noticeable. There was no indication in these cases that there had been a primary elevation of the epithelium which then led to a removal of the prominent parts; on the contrary it must be assumed that the rubbing-off process preceded or accompanied the downgrowth and may have acted as a secondary stimulus, aid-

6. Loeb, L.: Arch. f. Entwcklngesch. d. Mechn. **6**:1, 1897. Kelley, R. W., and Loeb, L.: Anat. Rec. **74**:487, 1939.

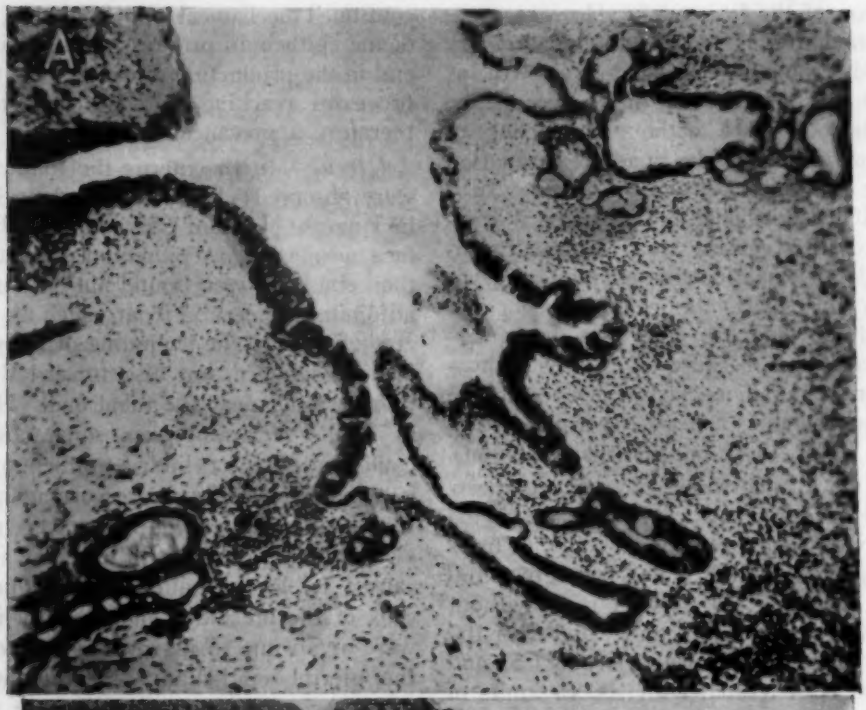
ing the action of the hormones. However, mechanical stimulation is not absolutely necessary for the production of epithelial downgrowth, as is indicated by the fact that in 4 mice active downgrowth took place without the action of such a factor; at least at the time of examination no evidence of this factor was observed. In 1 mouse combined hormonal and mechanical stimuli produced in a certain area abnormally enlarged basal nuclei and increased mitoses. Apparently in this place the cells had not yet developed the strength to push into the underlying stroma, which, because of its densely hyaline structure, offered marked resistance to the downward movements; instead, the twofold stimulation had led to these unusual manifestations.

Of the 8 CBA mice which showed precancerous or almost precancerous changes in the vagina or the cervix, 6 had been given 200 rats units of the estrogen, while only 2 had been given 100 rat units. Three of the 5 CBA mice without such growth processes had been treated with 100 rat units. Among the 3 mice of strains C57 and AKA, 2 had been treated with 100 rat units of the estrogen. There seems, then, to be a correlation between the amount of estrogen injected, the length of time during which it was administered and therefore also the age of the animals, on the one hand, and the development of these proliferative processes, on the other hand. As stated previously, the proliferative processes in the mammary gland were likewise most advanced in those animals which had been treated with 200 rat units of the estrogen; accordingly, in the group of animals showing strongly developed vaginal processes 3 had mammary carcinoma and 3 precancerous mammary changes, whereas only 1 in the group free of the more pronounced vaginal and cervical proliferations bore mammary carcinoma. In both the vagina-cervix and the mammary gland the strength of the stimuli acting on these two organs and the length of time during which they act help to determine the intensity of the resulting growth processes.

The growth processes observed in the vagina and cervix of these mice represent three different types. 1. Under the influence of an added exogenous estrogen the normal cyclic process produced by endogenous ovarian hormones is converted into a continuous proliferative change. 2. A combination of increased multiplication and migration of the cells of the cervical epithelium leads to invasion and substitution of the adjoining cylindric epithelium. 3. Invasion of the underlying stroma occurs; this also is induced by a combination of endogenous and exogenous estrogens with or without the cooperation of long-continued mechanical stimulation. If this activity of the epithelium progresses long enough, it may end in the production of cancer; the formation of processes reaching into the stroma represents therefore a precancerous state.

Uterus.—In the uterus the following changes were observed: 1. In 4 mice of strain CBA and in 1 mouse of strain C57 areas in which the surface or glandular epithelium consisted of more than one layer were found not only in the region adjoining the cervix, from which an ingrowth of epithelium into the beginning of the uterus regularly took place, but also toward the center of the uterus or near the tubal end. Here, as well as in the cervical region, squamous epithelium could undermine cylindric epithelium or destroy it by exerting sidewise pressure against it. Thus islands of squamous epithelium were noted within cylindric epithelium. Either these islands were caused by local proliferation and metaplasia of cells of the uterine cylindric epithelium or they were again due to ingrowth of the cervical into the uterine epithelium. In the latter case it would be necessary to assume that parts of the squamous epithelium which at first covered the uterine wall as a continuous layer degenerated secondarily and were replaced by the adjoining cylindric epithelium. There are indications that such degeneration may occur. Islands of squamous epithelium in the central or tubal portions of the uterus were found in 4 mice of strain CBA and in 1 mouse of strain C57 (31 per cent of the strain CBA mice and 38 per cent of the total mice used), while among 19 control mice of strain CBA this change was noted in only 3 (16 per cent); it seems, therefore, that the treatment of the mice with an exogenous estrogen intensified this change; these effects were noted in animals which received 100 rat units of the estrogen weekly as well as in those which received 200 rat units.

2. In 1 mouse of strain CBA and 1 of strain C57 uterine glands, lined by cylindric epithelium, penetrated the hyaline stroma to the inner muscle layer, broke through it in places and entered even the outer muscle layer. Mitoses were sometimes visible in the ducts of these glands, which were usually accompanied by some connective tissue strands. Cancerous changes were not seen, but the uterine glands were stimulated to slow proliferation following the long-continued action of estrogen; in none of the CBA controls was this change noted. However, in former investigations similar conditions had been observed in mice of other strains not subjected to injections of estrogen, especially in strains D and



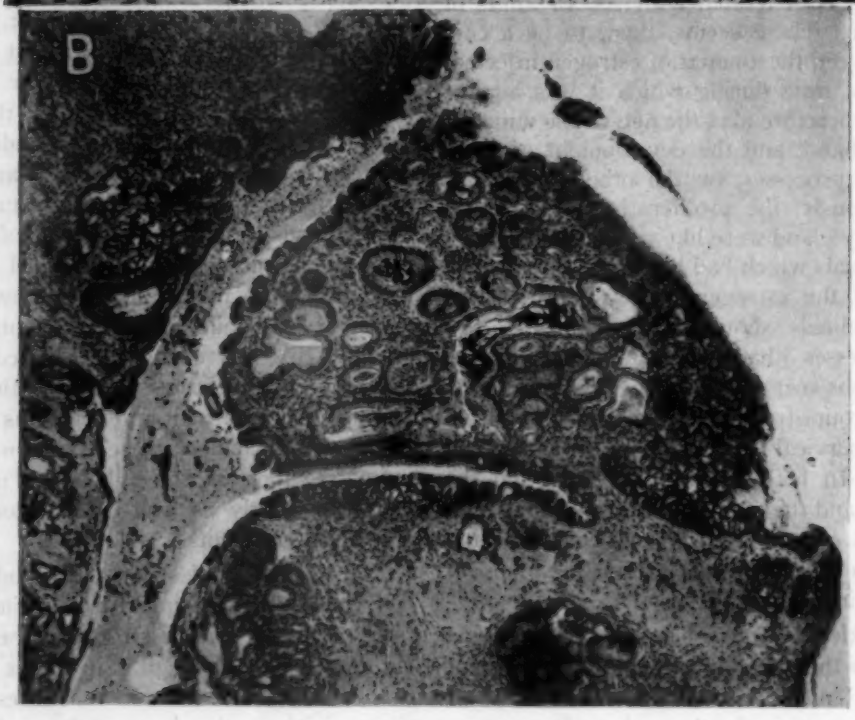


Fig. 1.—All the illustrations for this paper are from microscopic specimens obtained from mouse 13 of strain CBA, treated with weekly injections of 200 rat units of estrogen for seven months and eighteen days. This animal, which had been breeding, was 24¼ months old at the time of its death. Carcinoma developed in a mammary gland; in the cervix there were precancerous changes, and in the uterine surface epithelium and glands papillary changes had occurred.

A shows the papillary character and especially the fine secondary papillae in the surface epithelium of the uterus; they extend into the gland ducts without, however, reaching the fundi. In *B* fine secondary papillae cover the surface epithelium of a large primary papilla. One side of a gland duct is finely papillary. On the opposite side is seen a marginal section through a gland, the lumen of which is filled with fine papillae; the epithelial cells here are lower than they are in normal glands. On the other side of the uterine wall squamous epithelium has replaced a great part of the cylindric epithelium of a gland and is pushing aside the remainder of the cylindric epithelium.

C3H, but even here it appeared more frequently in mice receiving exogenous estrogen.[7]

3. In 3 mice of strain CBA papillary proliferations of a special kind were found in certain areas of the surface epithelium as well as in some uterine glands. The papillae were in close proximity to one another; they reached into the uterine lumen or into the lumens of the glands; the latter they filled almost completely. At the same time in certain places the glandular epithelium may have sent some processes to the outside. The epithelial cells which underwent this change became lower and at first showed a slight increase in mitoses, but subsequently, as the result of the formation of many papillae over a limited area, pressure was exerted on the connective tissue and the blood vessels which entered the papillae; in consequence of this secondary condition, the epithelial cells of the surface received in all probability a diminished amount of food, also of oxygen. Following these deprivations, the slight increase in mitotic proliferation ceased, and the epithelial cells underwent vacuolation. Glands showing papillae of this type did not penetrate the muscular layer of the uterus; as a rule, they were situated in the fibrillar or fibrous hyaline stroma of the mucosa, although in some instances they reached the periphery of

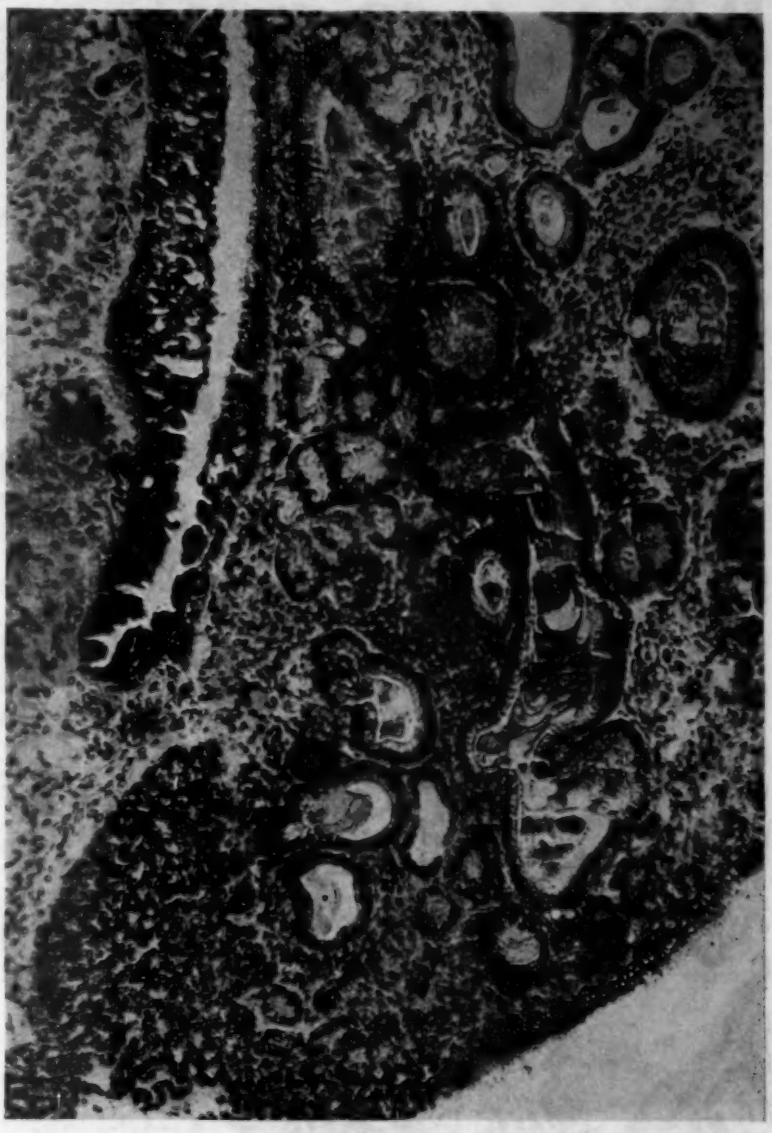


Fig. 2.—Higher magnification of a part of approximately the same field as that seen in figure 1 *B*.

7. Loeb, L.; Suntzeff, V., and Burns, E. L.: Am. J. Cancer **34**:413, 1938.

the inner muscle layer. Therefore, in this instance there had taken place to a certain extent a dissociation between the proliferative activity of these epithelial cells, which was slightly increased, and their movements, which were not noticeably increased. These changes were developed most strongly in the mouse given 200 rat units of the estrogen weekly for the longest time; they were of moderate intensity in a second mouse similarly treated for a shorter period and slight in a third that had been given injections of 100 rat units.

4. A proliferation of the peritoneal endothelium was observed in 2 mice of strain CBA, which received weekly injections of 200 rat units of the estrogen. There were areas in which the peritoneal endothelium had sent processes into the underlying connective tissue stroma after the endothelial cells had at first gradually enlarged and assumed a cuboidal shape and then had formed papillae; the processes penetrated deep into the wall of the uterus and here could make cell nests; on the other side of the papillary elevation and infiltrative growth the epithelium

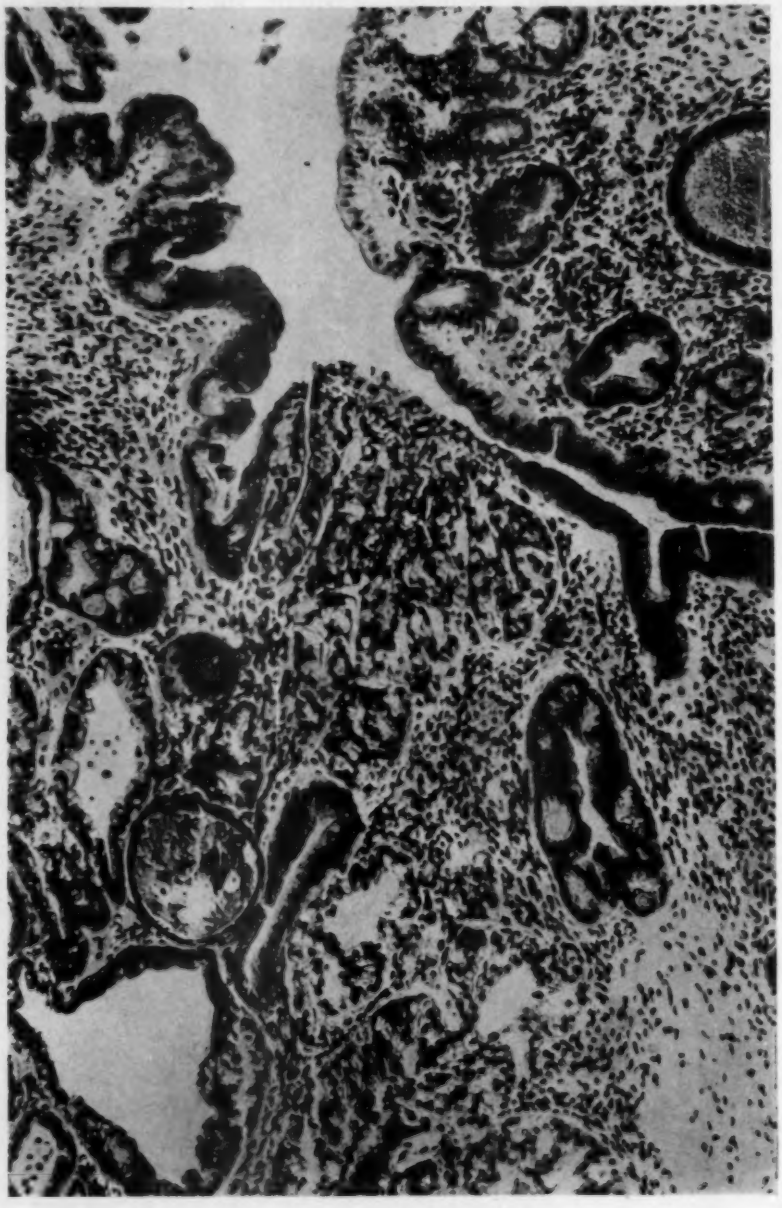


Fig. 3.—On one side the normal cylindric surface epithelium is seen; it connects with a gland, the lumen of which is filled with papillae; these papillae are covered with a low, vacuolated epithelium; likewise, the opposite side of the uterine surface epithelium is becoming lower.

gradually flattened out again and formed the typical lining of the peritoneum. One of these 2 mice had much dilated lymphatics in the area of activity of the enlarged endothelial cells, while in both mice abscesses were found in the upper portion of the uterus, and as the result of these inflammatory changes, adhesions of pancreatic tissue and uterine wall had formed at some distance from the papillary structures. It could be shown, however, that the papillary and invasive formations did not consist of adherent pancreatic tissue. It is probable that the uterine inflammatory changes helped to stimulate the peritoneal endothelium and that the injections of estrogen were also involved in these processes, because the latter were not found in the controls to which estrogen had not been administered.

These various uterine growth processes observed in old mice treated with an estrogen correspond to type II of the classification relating to the vagina and cervix. They all represent abnormal growth processes which are, however, as yet far removed from the transition to cancer. In addition there may be mentioned a deposit of large amounts of a hyaline material in the mucosa of the uterus and in the adjoining region of the cervix and vagina caused by the injections of large doses of the estrogen for longer periods. At first this material seems to be soft; then it becomes more dense in certain places; it is organized by connective tissue and blood vessels which invade it; at the same time new hyalin of the first kind is deposited. These processes, which have been described previously in younger mice,[8] took place also in the older mice studied in the present experiments. This hyaline substance stimulates the connective tissue and vessels of the mucosa to continued growth; it may injure certain glands surrounded by it and inhibit the outgrowth of glands or surface epithelium.

COMMENT AND CONCLUSIONS

In these experiments, in which mainly mice belonging to strain CBA, but in addition a few mice belonging to strains C57 and AKA, were used, the effects of long-continued and often repeated stimulation exerted by ovarian hormones, in particular by estrogen, on various tissues of the female sex organs, have been studied in older mice which had passed the reproductive period. One of the problems to which attention was given concerned the question whether in the animals advanced in age repeated exogenous hormonal stimuli would exert effects on tissues similar to those noted in younger animals, or whether in old animals reactions of a kind specific for old age might prevent efficiency of such exogenous stimuli. The observations recorded here lead to the following conclusions:

1. Distinct types of stimulation of growth may be observed under the influence of ovarian hormones acting on various tissues of older mice; (*a*) There is first intensification of normal growth processes, especially in the epithelium of the vagina and cervix, which did not return to the resting stage in these older experimental mice, in contrast to older control mice; the intensification of growth and of secretory processes in the mammary gland belongs to this category. (*b*) The second type is seen in abnormal growth processes that have not as yet reached the precancerous or the cancerous stage. These are represented by lateral expansive and invasive growth of the vaginal and cervical epithelium, by invasive growth of the uterine glands, which perforate the muscularis, by the peculiar papillary growth processes noted in some areas of the uterine surface epithelium and glands in certain mice, by the proliferative processes found in the peritoneal endothelium of some mice and lastly, in the ovaries of older mice, by the growth of ductlike structures which are derived from proliferating germ epithelium or medullary ducts; furthermore, the increased production of interstitial glands in the ovaries of these old mice may perhaps be included in these processes. (*c*) In the third type of stimulation of growth precancerous processes were observed in the mammary gland and in the vagina and cervix. In the former they were the result of intensification of the normal growth processes, and in the vagina and cervix they were apparently superimposed on the growth processes leading first to invaginations of the surface epithelium and then to the production of solid epithelial processes reaching into the stroma. The last-mentioned changes may lead to cancerous processes; these were found in the older experimental mice only in the mammary gland, but they may be expected to occur also in the vagina and cervix. Precancerous and cancerous reactions take place only if the intensity of tissue growth has reached an end stage, after these tissues have passed through several preliminary or preparatory stages of growth. During the latter stages processes of sensitization may take place which render the tissues more and more responsive even to stimulation of a nonspecific kind. The precancerous stage represents the transitional phase or series

8. Loeb, L.; Suntzeff, V., and Burns, E. L.: Am. J. Cancer **35**:159, 1939. Suntzeff, V.; Babcock, R. S., and Loeb, L.: ibid. **38**:217, 1940.

of phases which directly precede the acquisition of the new, cancerous tissue characteristics.

2. From these observations it follows that different kinds of tissues in the same individual or species respond to the same kind of stimulation with different degrees of intensity. This differential responsiveness is not due to unequal distribution of the stimulating substances in the vagina and cervix and in the mammary gland but to the inherence in each of these tissues of its own characteristic mode of response to these stimuli. The mammary gland is the most responsive. There follow in decreasing order the epithelium of the vagina and cervix and last the various uterine structures. These differences between different tissues are the same in the older animals used in these experiments as they were in the younger mice on which we have reported previously, and ultimately they are genetically determined.

3. There is usually associated with the increased intensity of growth increased migration, but the relative strength of these modes of response differs in different cases. When the cervical epithelium laterally invades the uterine epithelial structures, the migrating type of reaction is relatively prominent; on the other hand, when the papillary changes in the surface epithelium in the glands of the uterus occur, there is only a slight increase in proliferative processes but the motor activities remain dormant, the tissues being evidently not able to overcome the resistance of the surrounding stroma.

4. While ovarian hormones, and in particular estrogen, act as the essential stimuli that in the end bring about the transition from the normal to the cancerous state, secondary factors may contribute to their stimulating action. Under the conditions of these experiments two kinds of secondary factors were noted: (*a*) There is reason for the conclusion that the long-continued mechanical rubbing off of the keratin and of the upper layers of the squamous epithelium in the vagina may lead to regenerative processes which may intensify or accelerate the ingrowth of the epithelium into the connective tissue; this conclusion is in agreement with the observation that in general long-continued mechanical stimulation may end in cancerous transformation of the tissue. On the other hand, there is no reason for assuming that mechanical stimuli of a temporary kind, such as those represented by incisions, will have a lasting effect on the tissues; these cause merely a regenerative growth cycle of short duration and under certain conditions temporary growth processes of a specific kind, such as those leading to the formation of placentoma. Likewise, in the experiments of Overholser and Allen [56] the administration of estrogen combined with incisions of the cervix in monkeys did not lead to cancerous or precancerous lesions; but in 1 animal estrogen induced a precancerous change. (*b*) In proliferation of the uterine peritoneal endothelium, chronic inflammatory processes taking place nearby in the uterus may support the stimulation exerted by the ovarian hormones.

5. The tendency to undergo cancerization differs with different tissues within the same organism and is primarily determined genetically, notwithstanding the fact that every cell of an individual as far as it is known contains identical sets of genes. However, these experiments indicate in addition that in the same individual various tissues may all exhibit either strong or weak tendencies toward the development of growth processes, which, at first normal, gradually become intensified, then abnormal and ultimately cancerous. This correspondence in behavior of different tissues depends on the strength of the experimental growth stimuli acting on these tissues and on the length of time during which the various constituent parts of an individual have been exposed to the stimuli. Thus in mouse 13 of strain CBA adenocarcinoma was found in the mammary gland; in the vagina and cervix there were precancerous proliferations; in the uterus, islands of squamous epithelium in surface epithelium and in glands in various areas distant from the cervix. Papillary changes of an unusual kind were conspicuous in some parts of the surface epithelium as well as in certain glands. Other uterine glands which had not undergone this change perforated the muscle layers, and the peritoneal endothelium showed papillomatous proliferation and glandlike ingrowth into the stroma. This mouse had received weekly injections of the largest dose of the estrogen, 200 rat units, over the longest period; it was also the oldest mouse. The extraordinary development of these abnormal growth processes must therefore in all probability be attributed to the fact that in this animal the various tissues had been subjected to the strongest stimulation, with this factor acting on all parts of the organism irrespective of the constitutional factors characteristic of different tissues. If in the equation $H \times S = C$ H is constant, the intensity of the tissue proliferation and cancerization (C) will depend on the strength of the stimuli reaching these tissues. In other mice corresponding observations were made; in the AKA mouse, in which the stimuli acting on the various tissues were relatively weak, the growth processes in the tissues were likewise of low intensity. However, it is especially the

growth processes which are more intense, such as precancerous and cancerous changes, which seem to depend on a high threshold, i. e., on a greater intensity of stimulation, whereas the growth processes of lesser intensity require a lower threshold of stimulation, and these reactions seem to be less selective.

6. Our investigations do not indicate that the tissues in older animals are more liable to cancerization than those in younger animals. On the contrary, the decrease in growth energy noted with advancing age should counteract the tendency to cancerous change. Likewise, the denser nature of the stroma, by interfering with the nourishment of the tissues, should primarily have such an inhibiting effect; yet, as already pointed out, indirectly these conditions might favor the production of cancer. The main cause for the greater incidence of cancer in old animals, however, consists evidently in the accumulation in certain tissues with advancing age of stimuli to growth and in the sensitization which may occur as a result.

In addition to this accumulation of growth stimuli in the form of hormones and other substances there may be a development of secondary, nonhormonal factors which induce growth processes, and the association of the secondary with the primary, hormonal stimuli may in certain instances intensify the proliferative processes which ultimately lead to cancer. As such secondary processes we have recognized the long-continued removal of the upper layers of the vaginal epithelium by mechanical means and the chronic inflammatory processes in the uterus, which may help to induce papillary and invasive proliferation of the peritoneal endothelium.

Moreover, a virus-like substance, such as the so-called milk factor, may influence the production of mammary cancer in mice either by providing accessory stimuli or by increasing the responsiveness of the tissues to growth stimuli in a way similar to that of the genetic factors. The agent of chicken sarcoma may act directly as a growth and cancer promoting factor within the affected cells.

7. However, there are factors which oppose the effect of an accumulation of stimulations in certain tissues and organs. Formerly attention was called to the fact that stimulation of the thyroid gland of the guinea pig by anterior pituitary hormones is soon followed by a refractory period[9]; the same phenomenon is noted after stimulation of the thyroid gland by iodine.[10] In the mammary gland of the mouse hyaline changes in the stroma and certain atrophic processes in the fat tissue may lead to an injury of the glandular tissue causing a diminution in the reactions of the tissue to growth stimuli and thus diminishing the incidence of cancer in this tissue.[4c] In this investigation an additional example of such a cancer-inhibiting process has been observed. The papillary growth processes in the surface and glandular epithelium of the uterus interfere with the stroma and the blood vessels which carry nourishment to these epithelial tissues, and this results in self limitation of the growth processes.

8. There is evidence that in the old mice used in these experiments the exogenous estrogenic stimulus, which was added to the endogenous ones, was effective; both acted in the same direction on certain tissues, causing increased growth which led in some of these tissues to precancerous and cancerous changes. While for reasons stated, the number of mice which were available for the demonstration of these effects was restricted, nevertheless all the established data consistently point to this conclusion, and likewise our earlier investigations mentioned are in accordance with it.

9. As to the mechanism by which these stimuli lead to cancerization, it has been previously indicated that in all probability it consists in the production within the affected cells of intermediate products, autocatalytically propagating substances, which are the direct cause of increased cell activities and ultimately of cancer.[11] These stimuli would therefore induce chain processes which would give rise to the essential growth factors leading to cancerization.

SUMMARY

Various kinds of growth processes are described in the sex organs of older female mice which have passed the breeding period and which have been subjected to long-continued treatment with an estrogen in addition to the earlier action of the endogenous ovarian hormones. These proliferative changes are classified into (*a*) intensified normal growth processes, (*b*) abnormal growth processes, (*c*) precancerous and (*d*) cancerous changes.

Chronic mechanical stimuli, as well as long-continued inflammatory changes due to infection, may help to intensify some of the growth processes caused by hormones. There is no indication that acute regenerative processes have such an effect.

9. Loeb, L., and Friedman, H.: Proc. Soc. Exper. Biol. & Med. **29**:172, 1931.

10. Loeb, L.: Science **80**:252, 1934.

11. Loeb, L.: Science **43**:293, 1916. Loeb.[8a]

In the course of these cancerigenic stimulations conditions may develop which affect the stimulated tissues in such a way that their growth response is inhibited.

The various tissues of the secondary sex organs differ in their ability to respond to stimuli with proliferative changes which ultimately may lead to cancer. In the same individual, organ and tissue differences may be involved which are determined only indirectly by genetic factors; there exist also differences in the responsiveness of corresponding organs and tissues in different individuals and species, which are determined by genetic factors more directly and in a manner which is specific for these individuals and species.

It has been shown that within the same inbred strain or individual there probably exists, in addition to these differences in the mode of reaction of different tissues and organs to stimuli, a parallelism in intensity of the reactions of the various tissues and organs. This parallelism depends on the intensity of the hormonal stimuli, which differs in different individuals but which affects all the tissues in a given individual with the same relative strength.

These experiments indicate that the increase in the incidence of cancer with advancing age of individuals belonging to inbred strains is largely due to the accumulation of chronic stimulations of growth. As far as growth processes and cancerous processes are concerned, the tissues of older and those of younger organisms seem to differ only quantitatively in their reactions; the modes of their reactions seem to be similar.

It has, furthermore, been shown that the addition of an exogenous estrogen to the previously active endogenous ones may intensify certain growth processes initiated by the latter or may initiate other growth processes.

The photomicrograph in figure 1A was supplied by Dr. H. J. Blumenthal, and the photomicrographs in figures 1B, 2 and 3 by Mr. W. G. Elle of the Barnard Free Skin and Cancer Hospital.

Case Reports

ADDISON'S DISEASE WITH PARTIAL ABSENCE OF ADRENAL CORTEX AND GYNECOMASTIA

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We have found one report of a case of bilateral atrophy of the adrenal cortex associated with gynecomastia; the enlarged breasts were attributed to the cortical extract given the patient.[1] We now report a similar case.

A man aged 40, a former hosiery knitter, was admitted to the Evanston Hospital to the service of Dr. Lowell Snorf Oct. 2, 1942. He died eleven hours later. The complaints were cough productive of white mucus for one day, diarrhea for two days and generalized malaise and a sore throat for five days.

The onset occurred five days before when the patient became overheated and then chilled. The same evening he noted a mild sore throat, malaise and aching; diarrhea followed. The day before hospitalization he had a fever.

Four years previously he had acquired an unusually dark "sunburn" at the seashore. Shortly thereafter he began to suffer from dizzy spells and gastric disturbances. At first these were mild but eventually they became more severe and were associated with nausea, vomiting, progressive asthenia and loss of weight. A diagnosis of Addison's disease was made. Adequate amounts of salt and desoxycorticosterone acetate were administered. Two years later marked enlargement of the right breast was first noted. The patient's previous and family history were essentially without bearing on the case.

When examined the patient was stuporous. The systolic blood pressure was 70 mm. of mercury; the diastolic blood pressure could not be obtained. Respirations were 22 per minute. The skin was warm, moist and nut brown except for the genital region, which was deep brown-black. The lingual, buccal and gingival mucosae were marked by multiple dark brown pigmented areas. The right breast was enlarged and soft; the left breast was normal.

The breath sounds were almost completely suppressed in a small area medial and inferior to the right nipple, and on the left side of the chest medial and slightly superior to the inferior angle of the scapula. These areas were slightly hyporesonant and contained a few fine rales. The cardiac borders were within normal limits; the heart tones were faint and of varying intensity. The cardiac rate at the apex was 150 to 160.

The abdomen, the external genitals, the back and the extremities were normal.

The red blood cell count was 4,800,000; the hemoglobin content was 14 Gm. per hundred cubic centimeters; the white blood cell count was 6,900. Routine urinalysis gave negative results. The blood sugar was 83 mg. and the chlorides 237 mg. per hundred cubic centimeters. Cultures of the nasopharynx were negative, but a smear from the sputum contained many pus cells and a few gram-positive diplococci which did not type by the Neufeld reaction. The roentgenogram revealed mottled areas of increased density in the upper portion of the left lower pulmonary lobe.

A tentative diagnosis of addisonian crisis secondary to atypical pneumonitis, etiologic agent undetermined, was made. Large doses of adrenal cortical extract, intravenous injections of isotonic sodium chloride, sulfadiazine and symptomatic medications were given.

A necropsy was made ten hours after death. The body was that of an emaciated 40 year old white man. The weight was estimated to be 110 pounds (49 Kg.). The skin was deeply bronzed. Dark brown to black pigmented moles were on the face, the shoulders and the chest. The genital skin was dark brown. Superficial edema and lymphadenopathy were absent. The black hair of the scalp was abundant. Black punctate pigmentation of the tongue and diffuse dark brown to black pigmentation of the gingival margins and of the buccal mucosa opposite the molar teeth were present. A soft discoid mass, 9 cm. in diameter and elevated 3 cm., was palpated beneath the right nipple, but no fluid could be expressed.

The left pleural cavity contained 250 cc. and the right 100 cc. of clear serous fluid. Old fibrous adhesions were present along the posterolateral and over the apical portion of the left lung. The thymus was replaced by fibrofatty tissue except for scattered glandular remnants.

The left lung weighed 811 Gm. Multiple small gray thickenings were observed in the apical pleura. The lungs were edematous, and the dependent parenchyma exhibited nodular gray subcrepitant areas. Beta streptococci were obtained on culture. The right lung weighed 610 Gm. and was similar to the left.

The heart weighed 221 Gm. The left ventricular myocardium, which was dark brown and flabby, varied from 12 to 15 mm. in thickness; the myocardium of the right ventricle was 3 mm. thick. The endocardium was normal. The coronary arteries were normal except for an occasional small atheromatous intimal plaque. There was slight atherosclerosis of the aorta.

The liver and the gallbladder weighed 1,271 Gm. and were grossly normal. The spleen weighed 280 Gm. Its blue-gray capsule was studded with deep red, slightly elevated areas 1 to 2 mm. in diameter. The malpighian bodies were prominent, and the soft purple pulp scraped away with ease.

The left kidney (10.5 by 6.5 by 3.5 cm.) weighed 153 Gm. The capsule stripped with ease. The corticomedullary differentiation was indistinct. The right kidney (10 by 5.5 by 3.5 cm.) weighed 138 Gm. and was similar to the left. The renal pelves and ureters were grossly normal.

No definite adrenal glands were found in the normal or usual ectopic anatomic sites. The normal sites were occupied by fatty tissue which contained indefinite areas of dark brown, friable amorphous substance.

The periaortic lymph nodes extended partially into the normal location of each adrenal gland and were enlarged up to 3 by 3 by 1 cm. Each, however, was discrete and well encapsulated. Their sectioned sur-

From the departments of pathology and medicine of the Evanston Hospital, Evanston, Ill., and Northwestern University Medical School, Chicago.

1. Edwards, R. A.; Shimkin, M. B., and Shaver, J. S.: J. A. M. A. **111**:412, 1938.

faces everted to reveal a pale tan cortex and a pink-tan medulla.

The urinary bladder, the prostate gland and the right testicle were grossly normal; the left testicle was not examined.

The right breast (fig. 1) showed hyperplasia of ducts, acini and interstitial connective tissue.

The grossly pathologic areas of the lungs revealed interstitial inflammatory changes with a preponderance of large mononuclear cells.

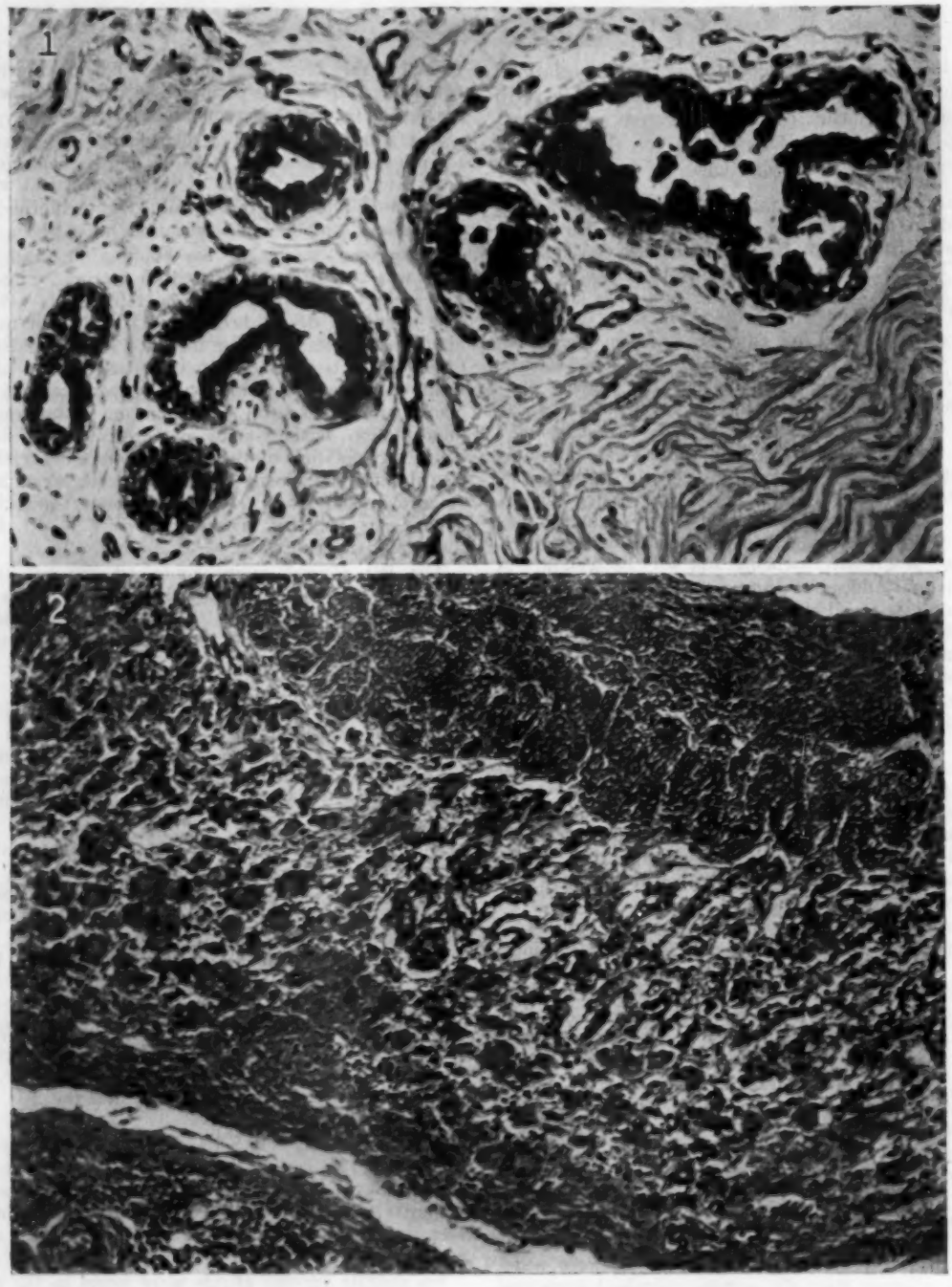

Fig. 1.—Hyperplasia of the right breast; × 96.

Fig. 2.—Section through the site of an adrenal gland; × 96.

The essential microscopic observations are described. The basal layer of the gingival epithelium was heavily pigmented, and some subepithelial areas were infiltrated by large mononuclear cells.

Serial sections through the fatty tissue of each adrenal site revealed extreme loss of all parenchymal components (fig. 2). Only an occasional cell cluster remained in the cortex; the medullary cells were more

abundant. Some of the cells of the zona reticularis were hypertrophied. Small round cells infiltrated the gland. The adjacent ganglions of the sympathetic nervous system were normal.

The periaortic lymph nodes contained oleophages surrounded by giant cells. Edema, slight hyperplasia of littoral cells and one calcareocaseous tuberculous nodule were seen.

COMMENT

Wells [2] reported a case of selective necrosis of the adrenal cortex in a woman who had been treated for pemphigus with germanin. Similar lesions were produced in laboratory animals by administration of germanin, and thus Wells was led to believe that necrosis of the adrenal cortex may be due to the action of some drug or chemical substance in persons with a particular idiosyncrasy.

2. Wells, H. G.; Humphreys, E. M., and Work, E. G.: J. A. M. A. **109**:490, 1937.

Duffin [3] recently reported several cases in which the adrenal lesions were similar to those we have described. He pointed to Wells's [2] suggestion that the adrenal lesions may be due to necrosis rather than atrophy. It is noteworthy that Duffin emphasized hyperplasia of lymphoid tissue similar to that seen in the periaortic nodes in the present case.

SUMMARY

Addison's disease developed in a man aged 36. He was treated by dietary and partial substitution therapy. Two years later an enlarged right breast was noted, and this enlargement persisted. At the age of 40 this man died in an addisonian crisis precipitated by atypical pneumonitis, the etiologic agent of which remained undetermined. The necropsy revealed almost complete absence of the adrenal cortex and hyperplasia of the right breast.

3. Duffin, J. D.: Arch. Path. **35**:649, 1943.

ARACHNOIDAL FIBROBLASTOMA (MENINGIOMA) WITH METASTASES TO THE LIVER

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The rare occurrence of extracranial metastases from a primary meningeal tumor justifies reporting another case. The variations among the reported examples prompt a brief critical review.

REPORT OF A CASE

A 41 year old Chinese was admitted to the Boston City Hospital on Nov. 26, 1940. For two months he had been mentally dull and for one month drowsy and weak. The week before hospitalization he had kept himself locked in a room.

When examined he was drowsy, disoriented and uncooperative, often not answering questions. His body was emaciated and dehydrated. His pupils reacted sluggishly. There was bilateral papilledema. Swallowing was difficult. Reflexes were generally hyperactive. Subsequently noted were increased muscular rigidity, decreased voluntary activity and generally unfavorable signs.

Increased pressure of the cerebrospinal fluid was demonstrated by lumbar punctures. Flattening and narrowing of the temporal horns and no filling of the anterior horns were shown by ventriculography.

On the fourteenth day a tumor occupying the greater part of the anterior fossa was surgically exposed. Following extensive removal of the tumor, the patient's blood pressure fell quickly, and respiration ceased.

The surgical specimen consisted of eight fragments, weighing 225 Gm., mostly of homogeneously firm rubbery gray tissue and partly of tissue resembling cerebral gray and white matter. Its histologic appearance is described in the report of microscopic observations.

Autopsy.—The subject was small and wasted. The main pathologic changes were in the cranial contents and the liver. The brain weighed 1,260 Gm. In each frontal pole was a crater with a floor of white matter and an edge of flattened rolled-in gyri. From the slightly larger right crater there was displacement to the left of the falx and the neighboring cerebral tissue. Ragged gray tumor tissue remained firmly attached to the cranial dura of the anterior fossa. The dura, however, was no more firmly bound to the cranium than usual, and there was no evidence of invasion of the underlying bone. The orbital plates of the frontal bones were thinned, but the calvarium elsewhere was of the usual thickness.

The liver weighed 880 Gm. The surface was brick red and smooth. Scattered through the lobes were eight spherical firm opaque gray masses, measuring from 0.3 cm. to 3.0 cm. in diameter. The intervening tissue was brown-red and possessed the usual consistence and lobular markings.

Microscopic Observations.—The intracranial tumor was composed of intermingling patterns varying with the shape and the interrelationship of the cells and with the quantity of stroma. Round to oval cells were closely packed in clusters and sheets with a scant network of collagen and reticulin. Elongated cells, also with little intercellular substance, formed interwoven bundles, or swept among the clusters of plumper cells. Heavier collagenous strands bounded some of these formations or were present within them, breaking them up irregularly. Small whorls of curved narrow cells arranged concentrically around a few round cells were abundant. Larger whorls with varying combinations of round and elongated cells were less numerous. In some areas the stroma was dense, forming branching masses that divided the cellular tissue into islands.

The cells generally had pale cytoplasm and indistinct borders. Their nuclei contained finely divided, deeply staining chromatin and distinct small nucleoli. In many nuclei were nongranular portions resembling vacuoles. Multinucleated cells were numerous. Occasional mitotic figures were present. Fibroglial fibrils were demonstrated in the stroma but not among the tumor cells. Of additional interest in the areas containing the dense collagenous masses were nests of branching cells with an abundance of neuroglial fibrils.

Blood vessels were plentiful in all parts of the tumor, yet in the more cellular regions cells midway between vessels were poorly defined or were replaced by hyaline material.

Where tumor opposed cerebral tissue the separation was complete, a thin layer of fibrous tissue and blood vessels intervening. The tumor ramified into the dura, in places splitting it completely and reaching its outer surface.

The tissue in the hepatic nodules resembled the intracranial tissue closely, having similar cells growing in sheets, bundles and whorls. The network of collagen and reticulin was more uniformly distributed through these formations. The dense fibrous masses and the neuroglial nests were lacking. The lobules of the liver adjacent to the tumor masses were compressed. Small portions were pinched off by expanding buds of neoplastic cells at the periphery of the masses and isolated within the tumor. A sharp separation did not exist between the tumor and the liver such as was present between the tumor and the cerebrum.

The diagnosis was arachnoidal fibroblastoma with metastases to the liver.

REPORTS OF MENINGEAL TUMORS WITH EXTRACRANIAL METASTASES

Certain neoplastic masses in abdominal or thoracic organs appearing coincidentally with diffuse meningeal growths have been either originally termed [1] or later cited [2] as metastases.

From the Mallory Institute of Pathology, Boston City Hospital.

1. Westphal, A.: Arch. f. Psychiat. **26**:770, 1894. Klebs, E.: Die allgemeine Pathologie, Jena, Gustav Fischer, 1889, vol. 2, p. 118.

2. Eberth, C. J.: Virchows Arch. f. path. Anat. **49**: 51, 1870. Olivecrona, H.: ibid. **217**:161, 1914. Lindner, E.: Ztschr. f. Heilk. **23**:118, 1902.

According to present concepts of tumors, however, it is unlikely that any of these extracranial masses had their origin in the cranial cavity.[3] The report of Derevici, Ionescu and Smilovici[4] and that of Pendergrass and Wilbur[5] are not considered pertinent, because in the former the abdominal masses were inadequately described and in the latter the pulmonary mass was visualized by roentgenogram only. The earlier surgically removed tissue of the case recorded by Cushing and Eisenhardt[6] was typically that of meningioma, but the final, metastasizing tumor did not match any recognized type of primary meningeal tumor.

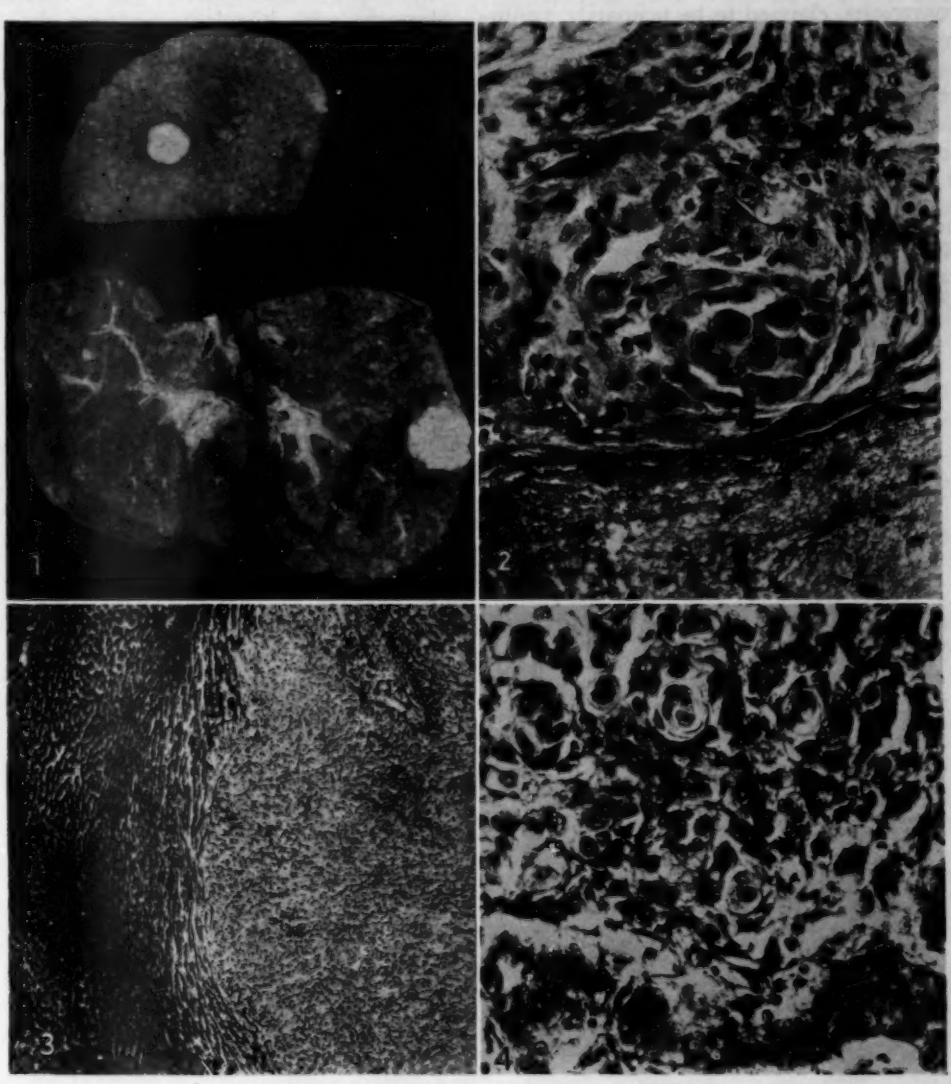

Fig. 1.—Gross appearance of cut sections of liver with nodules of metastatic arachnoidal fibroblastoma.

Fig. 2.—Section of the primary arachnoidal fibroblastoma, cerebrum and intervening vascular fibrous tissue. Phloxine and methylene blue stain; × 305.5.

Fig. 3.—Section of a nodule of the metastatic tumor in the liver, showing compression of the adjacent tissue of hepatic lobules, as well as the manner of extension of the tumor into these lobules. Mallory's aniline blue collagen stain; × 50.

Fig. 4.—Higher power of an area at the junction of tumor and liver, showing characteristic whorls of arachnoidal cells. Masson's trichrome stain; × 305.5.

Jurow[7] reported pulmonary metastases from a meningeal tumor in which microscopically patterns of cells and intercellular substance charac-

3. Nelson, A. A.: Am. J. Cancer **28**:1, 1936. Willis, R. A.: J. Path. & Bact. **47**:253, 1938.

4. Derevici, M.; Ionescu, E. I., and Smilovici, L.: Bull. Soc. roumaine de neurol., psychiat., psychol. et endocrinol. **18**:14, 1937.

5. Pendergrass, E. P., and Wilbur, D. L.: Arch. Neurol. & Psychiat. **19**:437, 1928.

6. Cushing, H., and Eisenhardt, L.: Meningiomas, Springfield, Ill., Charles C Thomas, Publisher, 1938, p. 692.

7. Jurow, H. N.: Arch. Path. **32**:222, 1941.

teristic of focal proliferations of arachnoidal cells were numerous. In the first of the 3 metastasizing tumors reported by Russell and Sachs [8] these arachnoidal designs were again evident.

Some primary meningeal tumors resemble morphologically types of tumors that may arise in other parts of the body. Examples of metastasizing fibrosarcoma, claimed to be primarily meningeal, are described by Russell and Sachs [8] in their second and third case reports and by Brandt [9] in his report of coexisting meningeal and hepatic masses.

The only other examples of extracranial metastases from primary meningeal tumors found are 2 diagnosed as melanomas reported by Foot and Zeek [10] and Ehnmark and Jacobowsky.[11]

8. Russell, W. O., and Sachs, E.: Arch. Path. **34**: 240, 1942.

9. Brandt, M.: Verhandl. d. deutsch. path. Gesellsch. **27**:39, 1934.

SUMMARY

A meningeal tumor with metastases to the liver was diagnosed as arachnoidal fibroblastoma. Two other cases of distantly metastasizing fibroblastoma with histologic features characteristically arachnoidal have been reported (Jurow [7]; Russell and Sachs [8]). Three cases of fibrosarcoma (Russell and Sachs [8]; Brandt [9]) and 2 of melanoma (Foot [10]; Ehnmark and Jacobowsky [11]) complete the list of reported primary meningeal tumors with extracranial metastases.

10. Foot, N. C., and Zeek, P.: Am. J. Path. **7**:605, 1931.

11. Ehnmark, E., and Jacobowsky, B.: Upsala läkaref. förh. **31**:565, 1926.

UNRUPTURED ANEURYSM OF THE BASILAR ARTERY

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Aneurysm of the cerebral vascular system is not infrequent, and the literature on it is extensive. McDonald and Korb[1] have compiled a series of 1,125 cases of intracranial aneurysm, defining the particular vessel and the comparative frequency. In 48 per cent the aneurysm was located on the internal carotid artery; in 15 per cent, on the anterior communicating artery, and in 28 per cent, posterior to the internal carotid artery. In 862 cases the aneurysm had ruptured, and splitting down this figure to the individual arteries one finds that the ruptured aneurysm was on the internal carotid artery in 48 per cent of these cases, on the anterior communicating artery in 17 per cent and posterior to the internal carotid artery in 24 per cent. This is quite in keeping with the various frequencies of occurrence. Of 143 listed cases of aneurysm of the basilar artery, rupture occurred in 89. Sixty-three per cent of the entire series presented concomitant vascular disease, while 36 per cent were listed as congenital. Syphilis seemed to play a minor role as an etiologic agent.

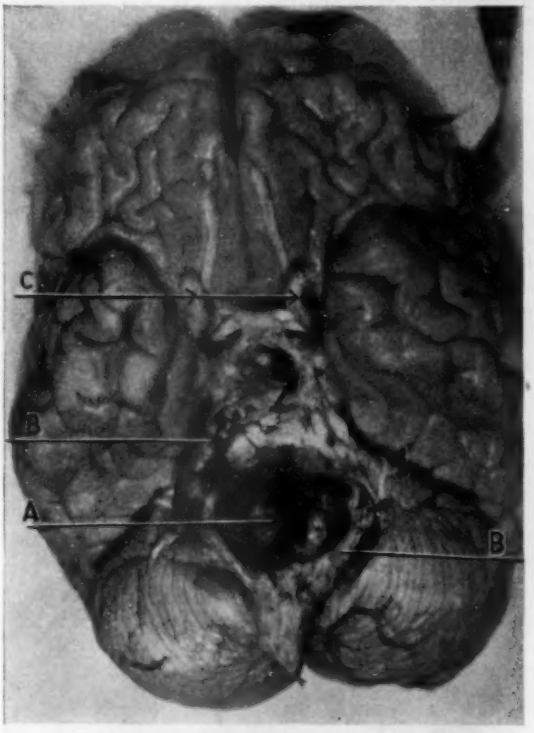

Fig. 1.—This defines the aneurysm (*A*) of the basilar artery (*B*) situated above the fusion of the cerebral arteries and at the pontomedullary sulcus. The sclerotic internal carotid arteries (*C*) are shown.

Clinical diagnosis is not easy, as it depends for the most part on physical signs and symptoms which may be indefinitely defined through pressure on neighborhood structures and an increase of intracranial pressure. Roentgenologic findings have played a recessive role because of the wide variations of density in the bone and soft tissue which must be traversed by the roentgen ray. Sosman and Vogt[2] have emphasized two roentgenologic findings of contributory importance in diagnosis. Should the wall of the aneurysm carry calcific deposits or the adjacent bone be eroded, the presence of aneurysm may be established or may be strongly supportive of clinical findings.

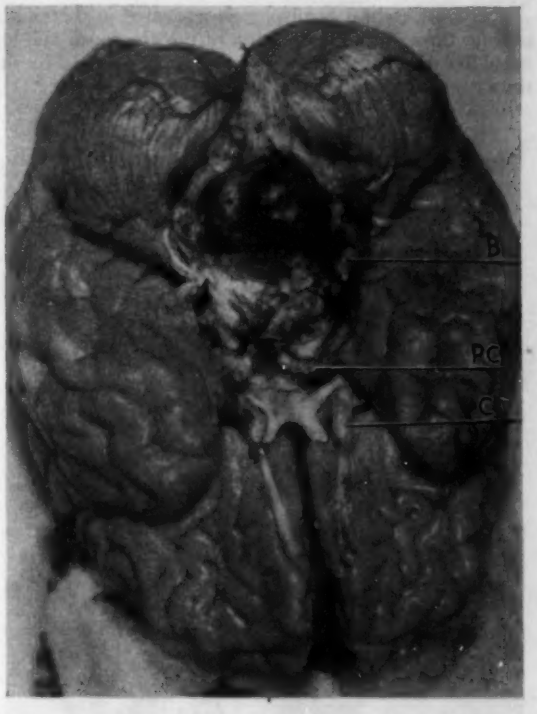

Fig. 2.—Same as figure 1 but defining better the atherosclerotic basilar artery (*B*) and the fluted posterior cerebral artery (*PC*). The posterior communicating artery on the right side is absent and that on the left is small. The smaller nutrient branches are obliterated. *C* indicates the carotid artery.

The causes of cerebral aneurysm are indefinitely defined, as are those of aneurysm of the

1. McDonald, C. A., and Korb, M.: Arch. Neurol. & Psychiat. **42**:298, 1939.

2. Sosman, M. C., and Vogt, E. C.: Am. J. Roentgenol. **15**:122, 1926.

arterial system in general. Sands [3] enumerated at least eight actually contributory causes for subdural hemorrhage, including ruptured aneurysm in the list. No insinuation of an aneurysmal tendency reflecting variations in the histologic anatomy of the walls of blood vessels either of the brain or of the systemic distribution has been made in the literature, and no concept such as that of a predilection of syphilis for producing aortitis and aneurysm of the aortic arch has been carried to the cerebral blood vessels even though meningovascular syphilis is readily accepted.

Many of these cases of aneurysm were undiagnosed till rupture occurred or until the unruptured aneurysm was encountered at postmortem examination. This paper reports a case of aneurysm of the basilar artery without rupture which yet was believed to be the cause of death because of its position and pressure on vital centers, i. e., malignancy by position.

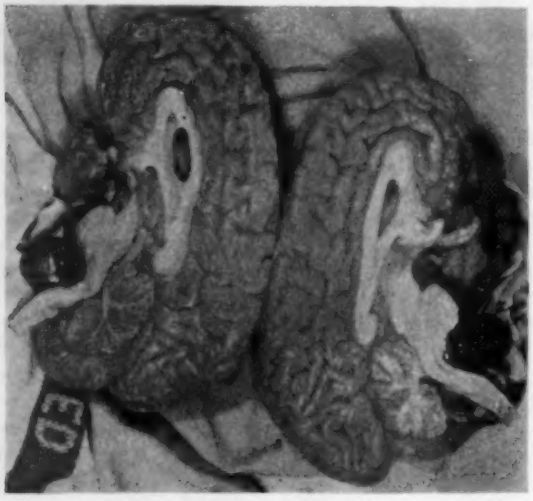

Fig. 3.—Cerebral hemispheres separated in the midline passing through the midportion of the aneurysm and laid open like the pages of a book. The aneurysm, in situ, shows a central blood clot and canalization.

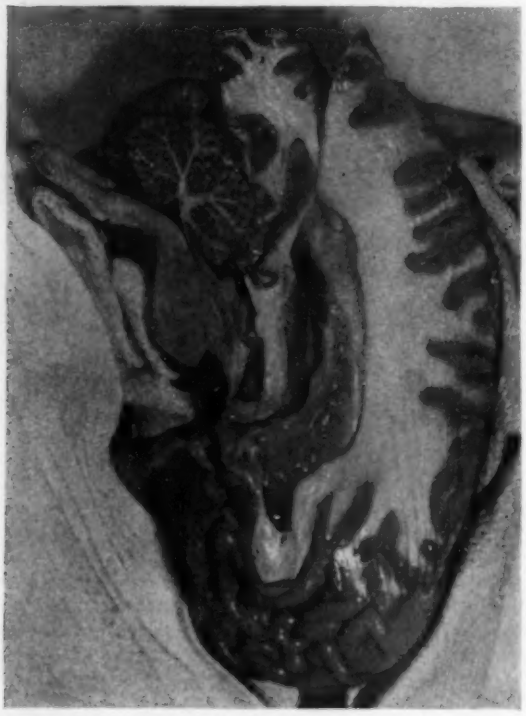

Fig. 4.—Same as figure 3 with removal of the aneurysmal sac, defining the results of pressure and cupping of the spinal cord.

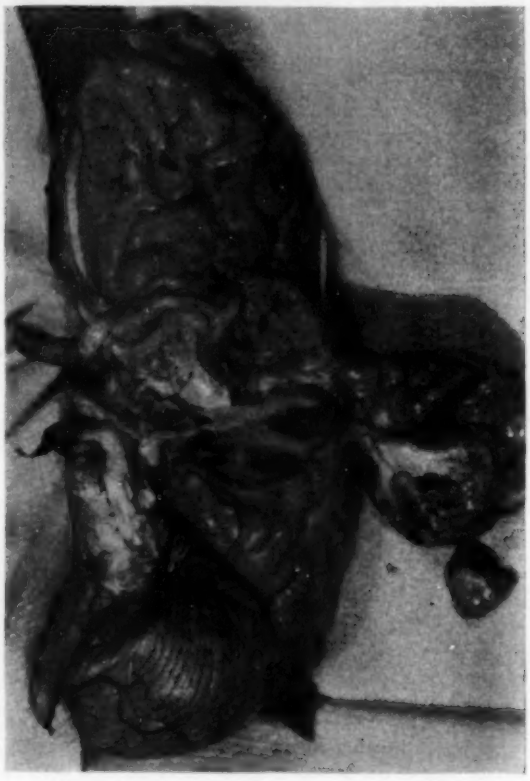

Fig. 5.—Further display of the sclerotic middle cerebral and internal carotid arteries.

The patient gave a history of good health until about a year previous to hospitalization when he noticed difficulty in recognizing and defining moving objects. Six months later he noticed double vision and some three months later swelling and disturbance of movement on the right side of the tongue, difficulty in swallowing with numbness of the right side of the face and frequent dizzy spells without a history of falling. He had noticed mental hebetude and difficulty in concentration. The blood pressure ranged in the neighborhood of 160 systolic and 120 diastolic without evident cardiac abnormality or complaint. Special examination of the eyes revealed normal internal and external structures of each eye and normal fundi. There was no impairment of movement. The patient carried his head slightly to the left, the Romberg sign was present, and there was a glide to the left in walking, which was exaggerated

3. Sands, I. J.: Arch. Neurol. & Psychiat. **40**:793, 1941.

when the eyes were closed, with a tendency to fall to the left. The pupils were small, the right larger than the left; the arc of accommodations was small; there was bilateral nystagmus on upward rotation and extremes of right and left movement. The tongue protruded to the left. The triceps reflexes were exaggerated, more on the left. The knee and ankle jerks were exaggerated, with ankle clonus. Babinski's reflex was noted on the right and the Allen-Cleckley [4] sign on the left. Sensation to light touch and pinprick was diminished on the right side of the face and forehead. Cardiac examination revealed absence of precordial distress and of respiratory disturbance. There was a history of fairly frequent occipital headache. The electrocardiogram showed a normal tracing. The roentgenogram was essentially negative. The results of chemical and serologic examination of the spinal fluid were essentially negative. The initial pressure was 108 to 266 on right Queckenstedt test and to 230 on left. The condition of the patient progressed continuously and steadily downward, the agonal changes being those of respiratory failure with mucous plugging of the larger bronchi and paralytic arrest of breathing.

Postmortem examination of the brain demonstrated an unruptured fusiform aneurysm of the basilar artery lying transversely across the brain stem in the sulcus immediately below the pons. The vertebral arteries presented the usual fusion forming the first portion of the basilar artery, which was displaced to the right and then abruptly curved to the left continuing into the aneurysmal sac to the opposite side of the cord and again curving abruptly to the left and continuing diagonally upward to the curve forming a good right posterior cerebral artery but a poor left posterior cerebral artery. The usual nutrient branches given off along this section were completely obliterated. The right posterior communicating branch forming the circulus arteriosus was patent, while the left was obliterated. The anterior inferior cerebellar arteries were obliterated, while the posterior ones were patent. The internal carotid and the anterior, middle and posterior cerebral arteries presented advanced atherosclerosis with no occlusion.

The sagittal section of the brain passed through the midportion of the aneurysm revealing the vascular wall intact, surrounding a canalized blood clot, the older former part of which was fused to the inner wall of the sac. The canalization was patent and complete and was filled with uncoagulated blood. The brain tissue offered no areas of softening. The posterior inferior surface of the pons carried pressure cupping. The medulla oblongata presented definite cupping and pressure compression over both olivary eminences and the area of the pyramidal decussation. The nerve filaments from the facial through to the accessory nerve were small and threadlike. Further observations were essentially negative for gross pathologic change of the brain. The vascular system in general presented moderate atherosclerosis without accentuation in the coronary, splenic and renal arteries and the lower portion of the abdominal aorta. The lungs presented definite edema and plugging of the bronchi with a mucous serous exudate.

Death is attributed to respiratory paralysis due to a fusiform aneurysm of the basilar artery with pressure over the area of the respiratory center.

4. Allen, L., and Cleckley, H.: New Pyramidal Sign of Great Frequency, J. Nerv. & Ment. Dis. **97:** 146, 1943.

PLASMOCYTOMA OF THE LUNG

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Recently Hellwig [1] in a comprehensive review of the literature collected 128 cases of extramedullary plasma cell tumor, including a single case of his own. McNamara and Rogers [2] have cited an additional case. The sites of election in 110 of the 128 instances reported by Hellwig were the upper air passages and the conjunctiva. Other sites were lymph nodes, the thyroid gland, the intestines, the kidney, the ovary, the spermatic cord and the skin. Bross [3] found a plasma cell tumor of the mediastinum in a 54 year old woman at necropsy, and Klose [4] observed a similar tumor of the pleura in a 61 year old man.

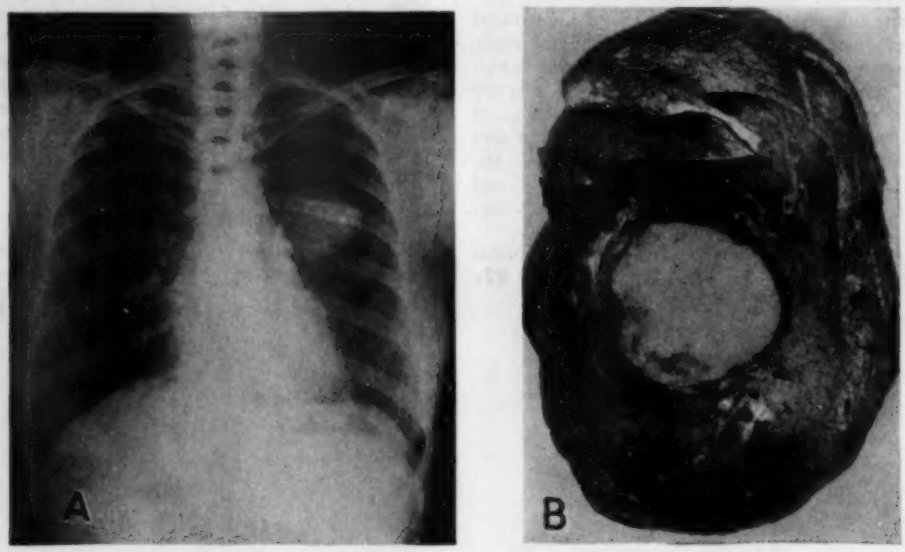

Fig. 1.—*A*, roentgenogram showing anterior-posterior view of a dense circumscribed shadow in the central and inner portion of the left pulmonary field.

B, cross section of the surgical specimen with the tumor in situ.

Differences of opinion have been expressed as to the nature of these extramedullary tumors. They are variously regarded as cancer or as granuloma secondary to chronic inflammation. Controversy also exists regarding the origin and the function of plasma cells.

The outstanding cytologic feature generally recognized is the abundance of plasma cells, which may be round, oval or polygonal. There is a large amount of basophilic cytoplasm, and the nuclei are situated eccentrically. The cells may often contain two, three or more nuclei, and the chromatin has a cartwheel arrangement. Giant cells may be present, as well as mitotic figures, which, as in other instances, do not necessarily indicate cancer. "Russell bodies," degenerated plasma cells containing hyaline acidophilic globules, may be seen.

A careful review of the literature failed to reveal an instance of plasmocytoma of the lung such as that presented by the patient here considered. However, in a personal communication Dr. F. W. Stewart, of the Memorial Hospital for the study of Cancer and Allied Diseases, informed us that he had observed 2 instances of solitary plasma cell tumor of the lung.

REPORT OF A CASE

An Italian housewife aged 30 had been in good health until November 1942, when, following a spontaneous abortion, she had a low grade fever for several weeks, unaccompanied by outspoken symptoms. Dr. F. A. Mastrianni, of Mechanicville, N. Y., referred the patient to Dr. E. A. Suss, superintendent of the Homestead Sanatorium at Middle Grove, N. Y., who made the diagnosis of tumor of the lung, and arranged for the patient to come to Ray Brook for operation.

On admission, March 28, 1943, the patient appeared healthy, well developed and in good nutrition. She felt quite well and had no complaints. The pulse, the res-

From the New York State Hospital for Incipient Pulmonary Tuberculosis.

1. Hellwig, C. A.: Arch. Path. **36**:95, 1943.
2. McNamara, W. L., and Rogers, R. J.: Arch. Path. **36**:89, 1943.
3. Bross, K.: Folia haemat. **45**:137, 1931; cited by Hellwig.[1]
4. Klose, H.: Beitr. z. klin. Chir. **74**:20, 1911.

pirations and the temperature were normal. The examination, including the pelvic organs, failed to show evidence of disease. The blood pressure was 120 systolic and 80 diastolic. Roentgenographic examination of the chest showed a round, sharply circumscribed, dense shadow, approximately 2 inches (5 cm.) in diameter, located in the upper and inner part of the left pulmonary field (fig. 1 *A*). Roentgenographic examination of the long bones and the skull revealed no abnormalities. The trachea and the major bronchi were normal in appearance on bronchoscopic examination. Examination of the blood and the urine, including the test for Bence Jones protein, gave no remarkable results. No sputum was available. The Mantoux test showed a positive reaction to 0.5 mg. of old tuberculin. The erythrocyte sedimentation rate was 70 mm. per hour by the Westergren method. The Wassermann test of the blood was negative. The diagnosis of tumor of the lung was confirmed; the nature of the tumor remained undetermined.

With the patient under general anesthesia, the upper lobe of the left lung was removed, the individual ligation technic being used. The left phrenic nerve was crushed to produce temporary paralysis of the diaphragm and thereby diminish the volume of the left hemithorax. The patient had an uneventful convalescence and was discharged four weeks later.

The specimen consisted of the upper lobe of the left lung, which after fixation in a 4 per cent solution of formaldehyde, measured 15 by 11 by 8 cm. The tissue showed no abnormality except for some congestion in the upper half of the lobe. Embedded in the medial aspect of the lobe there was a globular mass 5 cm. in diameter, which was well encapsulated. On the medial aspect this mass projected beyond the surface of the lung but beneath the pleura for several millimeters, just anterior to the bronchi and vessels at the hilus.

The mass was firm and resilient, the consistency being that of a uterine leiomyoma. It was definitely encapsulated at all points and was easily separable from the surrounding lobe. There was a small pedicle at the hilus, composed of areolar tissue and a few small vessels. The cut surface of the tumor was a light

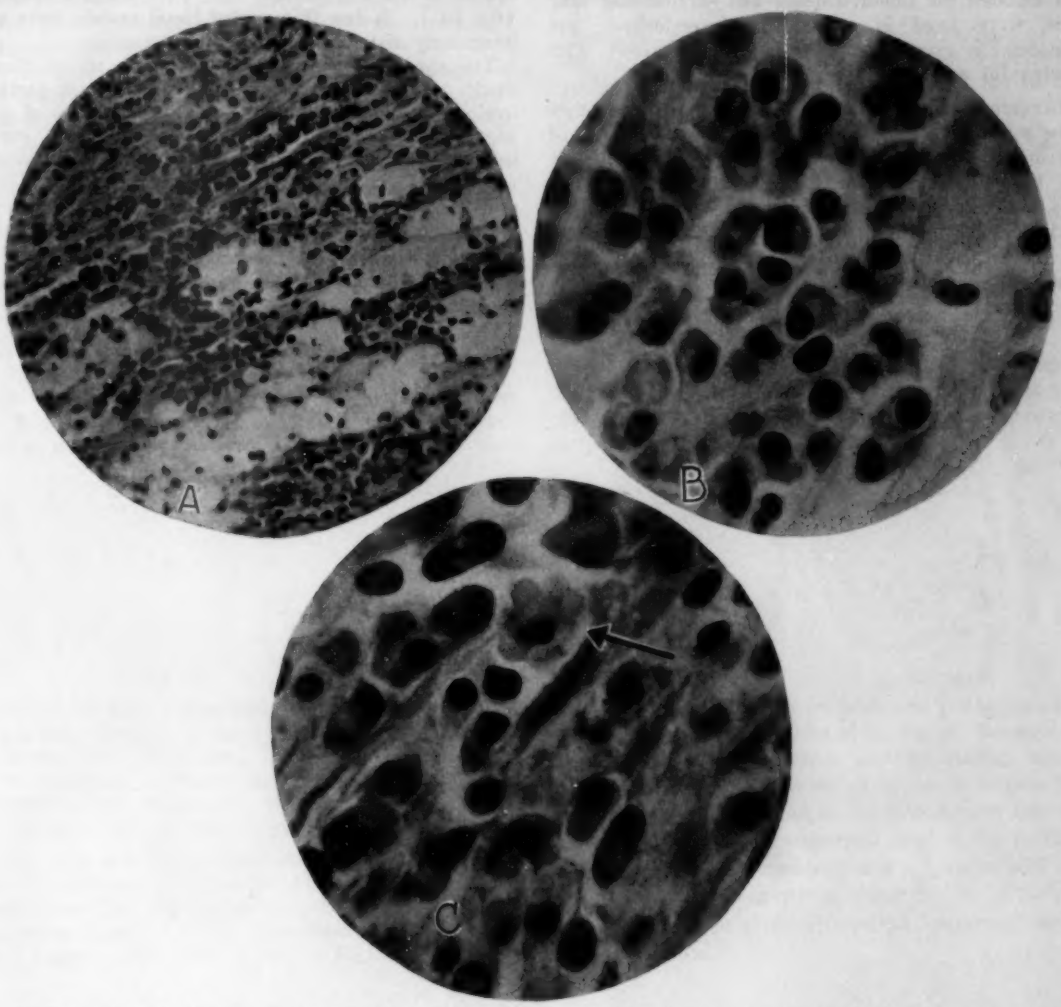

Fig. 2.—*A*, stained microscopic preparation (× 160)—a section of the tumor showing the plasma cells, mononuclear cells containing lipids, and lymphocytes.

B, stained microscopic preparation (× 1,000) showing details of the predominant plasma cells and the cartwheel appearance of the nuclei.

C, stained microscopic preparation (× 1,000) showing several refractile hyaline "Russell bodies" within a degenerating cell.

pearly gray for the most part, but scattered throughout were numerous small bright yellow areas of irregular outline, averaging 2 mm. in diameter. In one area were several small flecks resembling blood, averaging 2 mm. in diameter. The section surface was of uniform consistency except for irregularly interlacing fibrous strands. The fibrous capsule was firmly adherent to the tumor mass and did not evert from the section surface. No extension of the tumor beyond the capsule was made out. The lung tissue adjacent to the tumor was moderately compressed; otherwise it was normal in appearance (fig. 1 *B*).

Blocks of tissue, including complete transverse sections through the tumor, capsule and surrounding lung tissue, were fixed in solution of formaldehyde and embedded in paraffin. Sections were stained with hematoxylin and eosin.

Microscopic Observations.—The outstanding feature of the microscopic appearance was the predominance of cells indistinguishable from plasmocytes, with abundant dark basophilic cytoplasm, distinct round or oval cell walls, and eccentrically placed small round nuclei in which the chromatin granules were numerous, coarse and in typical cartwheel distribution (fig. 2 *B*). Occasional cells contained two or three such nuclei. The cells were uniformly well differentiated; mitotic figures were rare. The plasma cells showed no definite arrangement or grouping, but in many areas they were separated into cordlike clusters by the numerous fine connective tissue strands found throughout the tumor. Scattered throughout the tumor, but in greatest number just within the capsule, there were groups of lymphocytes, which in many areas showed arrangement into secondary lymphoid follicles. Within the substance of the tumor there were clusters of large mononuclear phagocytes. The foamy appearance of the cytoplasm of these was evidence of the lipoid content (fig. 2 *A*). Moderate numbers of the so-called Russell bodies, acidophilic hyaline droplets of varying size, were found throughout (fig. 2 *C*). A few thin-walled blood vessels were seen irregularly distributed throughout the tumor.

The capsule of the tumor was made up of fibrous tissue, and scattered among irregular bands of partially hyalinized connective tissue there were clusters of plasmocytes, which, however, did not appear to extend beyond the capsule. In some few areas blood vessels were found penetrating the capsule. There were compression of the surrounding air spaces and lymphocytic infiltration of the corresponding alveolar walls. No other abnormalities of the lung were noted.

The site of origin of the tumor was not determined.

Notes and News

Appointments.—John G. Kidd, associate member of the Rockefeller Institute of Medical Research, has been appointed professor of pathology at the Cornell University Medical College and pathologist at the New York Hospital.

Stuart A. Wallace, chairman of the department of pathology of the Baylor University College of Medicine, Dallas, Texas, has been appointed to the newly endowed Fulbright professorship of pathology.

Elizabeth Krauskopf Bushnell, adjunct professor of bacteriology at the University of South Carolina, has been appointed assistant professor of bacteriology at the University of Hawaii.

Nicholas W. Popoff has been appointed director of laboratories of the Genesee Hospital, Rochester, N. Y.

Awards.—The University of Toronto has awarded the Charles Mickle Fellowship for 1943 to Evarts A. Graham, Bixby professor of surgery at the Washington University School of Medicine, St. Louis, in recognition of "his discovery of a method of testing gallbladder functions by the use of certain organic compounds and the diagnosis and treatment of carcinoma of the lung."

The American Foundation for Tropical Medicine, Inc., has established an award for outstanding achievement in tropical medicine, to be known as the Richard Pearson Strong Medal for distinguished achievement in tropical medicine. It consists of a palladium medal with a cash honorarium of $500, the gift to the foundation of the Winthrop Chemical Company. The first award was presented to Colonel Strong at the annual meeting of the American Foundation for Tropical Medicine in New York on February 28.

Society News.—The scientific meetings of the American Association of Pathologists and of the American Association for Cancer Research will not be held in 1944.

Deaths.—W. W. C. Topley, outstanding English bacteriologist, immunologist and administrator, died Jan. 21, 1944, at the age of 57. He was the author of an "Outline of Immunity" and, with G. S. Wilson, of "The Principles of Bacteriology and Immunity," a standard work of reference. He was one of the first to study epidemiology experimentally. In 1942 he was awarded a Royal Medal of the Royal Society for his work on epidemiology and immunity.

William G. MacCallum, professor of pathology in the Johns Hopkins University School of Medicine since 1917 until his retirement in 1943, died on February 3 at the age of 69 years.

Book Reviews

Maurice Arthus' Philosophy of Scientific Investigation. Preface to De l'anaphylaxie a l'immunite, Paris, 1921. Translated from the French, with an introduction by Henry E. Sigerist. Foreword by Warfield T. Longcope. Pp. 26. Price 75 cents. Baltimore: The Johns Hopkins Press, 1943.

This book is a reprint from the *Bulletin of the History of Medicine* (**14**:366-390 [Oct.] 1943). In the introduction Dr. Sigerist gives a brief but impressive account of Maurice Arthus as teacher and experimental physiologist. The preface, which is ably translated, eloquently advocates the methods and principles of experimental research introduced by Claude Bernard, and practiced by Louis Pasteur and by Arthus himself. Arthus felt compelled to do all he could to pass these methods and principles on to his students and followers. His analysis of the experimental method, his insistence on thoroughness, independence and originality will guide and sustain the beginner in research. It is fortunate that "this unique piece of medical literature" now is easily available.

CORRECTIONS

In the article by Dr. Philip Levine entitled "Mechanism of the Isoimmunization by the Rh Factor of Red Blood Cells," in the February issue (ARCH. PATH. **37**:83, 1944), the first word in the second line of the last paragraph in the right hand column on page 88 should be "Rh-positive," instead of "Rh-negative."

Books Received

ORAL HISTOLOGY AND EMBRYOLOGY. Edited by Balint Orban, Foundation for Dental Research of the Chicago College of Dental Surgery, Loyola University School of Dentistry, Chicago. Pp. 342, with 262 illustrations, including 4 color plates. Price $6.50. St. Louis: C. V. Mosby Company, 1944.

The first two chapters deal with the development of the face, the oral cavity and the teeth. Then come chapters on the structure and development of the enamel, the dentin, the pulp, the cementum and the periodontal membrane, the maxilla and the mandible (alveolar process) and the gingival sulcus and the epithelial attachment; on the eruption and shedding of teeth; on the oral mucous membrane and glands; on the temporomandibular joint and the maxillary sinus, and on technical matters. At the ends of the chapters are paragraphs on the clinical bearings of the structures and processes described; also lists of well chosen references for further study. The illustrations, nearly all original, were selected as the best in the pooled illustrative material of the contributors. The 19 authors are members of the faculties of schools of dentistry and of oral and dental surgery in different parts of the country. Each chapter was drafted by a specially qualified author and critically discussed by the other authors. The chapters in the book represent the outcome of careful coordination of the views of all the authors. In this way "the major differences in concept were successively eliminated." The book will be of great value to students, dentists and all who study oral histology and embryology. It is a model of cooperation and coordination in multiple authorship.

TUBERCULOSIS IN THE UNITED STATES. Graphic Presentation. Prepared by the Staffs of the Division of Public Health Methods, National Institute of Health, and the Tuberculosis Control Section, States Relations Division, United States Public Health Service. Under the Direction of Carroll E. Palmer. Volume I: Mortality Statistics for States and Geographic Divisions by Age, Sex and Race. Price $1.50. New York: National Tuberculosis Association, 1790 Broadway, New York 19, 1944.

A TEXT-BOOK OF PATHOLOGY. Edited by E. T. Bell, M.D. Contributors: E. T. Bell, M.D., professor of pathology, B. J. Clawson, M.D., professor of pathology, and J. S. McCartney, M.D., associate professor of pathology, University of Minnesota, Minneapolis. Fifth edition. Pp. 862, with 448 engravings and 4 colored plates. Price $9.50. Philadelphia: Lea & Febiger, 1944.